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The Journal of Allergy

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Original Articles

CLINICAL AND SEROLOGIC STUDY OF THE RELATIONSHIP OF GIANT (AMBROSIA TRIFIDA) AND LOW (AMBROSIA ARTEMISIAEFOLIA) RAGWEED POLLEN*†

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NEW YORK, N. Y.

INTRODUCTION

IN THE eastern section of the United States the pollens of the giant ragweed *(Ambrosia trifida)* and the low ragweed *(Ambrosia artemisiaefolia)* are the most important factors producing autumnal hay fever. Cooke and Vander Veer,[1] in a series of 26 such cases, showed that those that reacted positively in direct skin test to one of these ragweed pollen extracts reacted similarly to the extract of the other pollen. With Spain[2] in a clinical study in which 800 cases were tested, similar skin reactions resulted with extracts of the two pollens. Brown[3] in a series of 31 autumnal hay fever subjects found similar skin reactions to the extracts of the two ragweed pollens. One group was given the usual series of prophylactic injections of one of these pollen extracts, and the remainder were treated with the other extract. After a period of treatment the group receiving the giant ragweed was given the low ragweed, and the group receiving the low ragweed was given the giant ragweed in equivalent doses. Since no constitutional reactions resulted, he concluded that the specific excitants of the two pollens were identical. Bernton[4] and Rappaport and Johnson[5] found differences in skin sensitivity with extracts of the giant and low ragweed pollens. Spain and Hopkins[6] in a clinical and serologic study of these two pollen extracts could demonstrate no difference in the excitants of the pollens.

Using the passive transfer method of Prausnitz and Küstner,[7] Coca and Grove[8] could demonstrate no difference in the active excitants

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†Read in abstract before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 28, 1934.

of these two ragweeds by means of the specific exhaustion of sensitized skin sites in normal persons. Moore, Cromwell and Moore[9] noted that passive transfer skin sites desensitized by giant ragweed were sensitive to a mixed ragweed extract. Walzer and Grove[10] using the Dale technic found that the uterine strip of the guinea pig, sensitized to either giant or low ragweed pollen extract, was equally reactive to both. They found that desensitization to either pollen extract rendered the uterine strip insensitive to both, and concluded that the active principle of these two species of ragweed was identical. Armstrong and Harrison[11] noted differences in cross fixation reactions between rabbit antiragweed serums and the two ragweed extracts Cromwell and Moore[12] found that the trypan blue reaction in ragweed sensitized rabbits showed a greater skin sensitiveness of the animals to the homologous than to the heterologous pollen extract. Precipitin reactions with the serums from these animals showed certain specificity, and the antigenic structure of the giant and low ragweed pollen was considered different.

Stull, Cooke and Chobot[13] tested the purified protein prepared from the giant and low ragweed pollens by the method of passive transfer. It was found that nine ragweed sensitive serums were desensitized by the pure protein from either the giant or the low ragweed when solutions of the protein in strength of 1,000 units[14] (0.01 mg. protein N) per c.c. were used. A small series of cases, therapeutically treated, were equally protected by the pure protein of either pollen, and at the end of the treatment period they were changed from one ragweed protein to the protein of the other ragweed without constitutional reactions. From these results the two pollens were considered biologically identical.

In 1931 a large group of autumnal hay fever patients in this clinic was treated with extracts prepared from the giant ragweed. These perennially treated cases were changed in 1932 from the old extract to the new extract on the basis of the protein nitrogen unit[14] without difficulty or constitutional reactions. In 1933 the new ragweed extract for this group was made from one-quarter of low ragweed and three-quarters giant ragweed pollen. When these patients were changed to this new extract on the protein nitrogen unit similar to that of the previous year, a number of constitutional reactions resulted. The symptoms in these cases began within an hour and in some cases within fifteen minutes, and consisted of generalized urticaria, asthma or vasomotor rhinitis. In some the symptoms were mild, in others very severe requiring several injections of epinephrine to control them. Check analyses showed that no error had been made in the standardization or dilutions, and, as the only difference from the previous year was the presence of the low ragweed, this study was undertaken to explain

this phenomenon in view of the fact that the weight of evidence, including our own, favored the conclusion that the allergic activity of the extracts of these two species of ragweed pollen was identical.

The work here reported will show in a series of patients, therapeutically treated with higher dosage, that the change in some cases could not be made from a giant ragweed extract to the same protein unit dose of low ragweed extract without producing constitutional reactions; although the reverse change could always be made without constitutional reactions. In a serologic study by more accurate quantitative methods with more dilute extracts of the giant and low ragweed, and more dilute solutions of their active proteins, slight differences in activity and marked differences in the serum neutralizing capacities of these two pollens were demonstrable.

CLINICAL STUDY

(A) Comparison of Giant and Low Ragweed Extracts Prepared From Pollens Collected in Different Seasons.

In May, 1933, four pollen extracts were freshly made from pure giant and pure low ragweed pollens collected in different seasons and carefully preserved. The extracts were all prepared from ethyl ether extracted pollen in alkaline-saline fluid.[14] The dilutions were made in buffered saline[15] on the protein nitrogen basis[14] (1 unit = 0.00001 mg. protein nitrogen). The analyses of these extracts are given in Table I.

TABLE I

RAGWEED EXTRACTS IN ALKALINE SALINE (5 GM. POLLEN PER 100 C.C.)

POLLEN	SYMBOL	YEAR COLLECTED	NITROGEN MG. PER C.C.		UNITS PER C.C.
			TOTAL	PROTEIN	
Low ragweed	LR-24	1924	0.56	0.24	24,000
Low ragweed	LR-32	1930-1932	0.70	0.35	35,000
Giant ragweed	GR-24	1924	0.46	0.20	20,000
Giant ragweed	GR-29	1929	0.76	0.30	30,000

These extracts were used in the treatment of a group of perennially treated patients. All patients were tested intracutaneously with 10, 100 and 1,000 protein nitrogen unit dilutions of the freshly prepared pollen extracts, and in none of the cases did the respective dilutions of giant or low ragweed give any determinable difference in the size or activity of the wheals produced. Inoculations were then given in increasing doses at weekly intervals. When the dosage had been reached as shown in column 1, Table II, a change was made to another extract at weekly intervals until the different extracts had been given.

Table II shows that all patients changed from GR-24 to GR-29 in equal doses and vice versa were free of constitutional reactions. When

Table II

Local and General Reactions After Changing Therapeutic Injections of Giant and Low Ragweed Extracts at Weekly Intervals

Case	Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later: Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later: Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later: Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later: Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.
1	GR-24	3000	++	0	GR-29	3000	+	0	LR-24	3000	+	+								
2	GR-24	2000	++	0	GR-29	2000	++	0	LR-24	2000	+++	++	GR-29	5000	+++	0				
3	GR-24	2000	+++	0	GR-29	2000	++	0	GR-29	2000	++	0	LR-24	2000	0	+				
4	GR-24	4000	+	0	LR-24	4000	++	++	GR-29	4000	++	0								
5	GR-24	1000	+	0	LR-24	1000	+++	+++	GR-29	1000	+	0								
6	GR-24	2000	+++	0	LR-24	2000	++++	+	GR-29	2000	+	0	LR-24	2000	++++	+				
7	GR-24	800	+	0	LR-24	800	++++	+++	GR-29	800	++	0								
8	GR-24	300	++	0	LR-24	300	+++	+	GR-29	300	++	0	LR-24	300	+++	+				
9	GR-24	1500	+++	0	LR-24	1500	+	++	GR-29	1500	++	0	LR-32	1500	++	0				
10	GR-24	600	+++	0	LR-24	600	++++	+	GR-29	600	+	0	LR-24	600	++++	0				
11	GR-24	400	++	0	LR-24	400	++++	++	GR-29	400	++	0	LR-24	400	++++	+				
12	GR-24	2000	+	0	LR-24	2000	+++	+	GR-29	2000	0	0	GR-24	4000	+	0	GR-29	7000	++	0
13	GR-24	2000	+++	0	LR-24	2000	++++	+	GR-29	2000	++	0	LR-24	2000	++	0	GR-29	5000	++	0
14	GR-24	1200	+	0	GR-29	1200	+	0	LR-24	1200	+++	0								
15	GR-24	5000	++	0	LR-24	5000	++++	+	GR-29	5000	0	0	LR-24	5000	+++	0				
16	GR-24	5000	+++	0	LR-24	5000	++++	0	GR-24	7000	+	0	GR-29	7000	0	0	LR-24	7000	+	0
17	GR-24	5000	++	0	GR-29	5000	+	0	GR-24	5000	+	+								
18	GR-24	5000	++	0	LR-24	5000	++++	0	GR-24	7000	+++	0	GR-29	7000	++	0	LR-24	7000	++	0
19	GR-24	1500	+	0	GR-29	1500	+	0	LR-24	1500	+	0								
20	GR-24	400	++	0	LR-24	400	++++	0	GR-24	600	+++	0	GR-29	600	++	0	LR-24	600	+++	0
21	GR-24	2000	++	0	GR-29	2000	++	0	LR-24	2000	+++	0								
22	GR-24	2500	++	0	LR-24	2500	++++	0	GR-24	4000	++	0	GR-29	4000	+++	0	LR-24	4000	+++	0
23	GR-24	3000	++	0	GR-29	3000	++	0	LR-24	3000	++	0								
24	GR-24	2000	+	0	GR-29	2000	+	0	LR-24	2000	++	0								
25	GR-24	1500	+	0	LR-24	1500	+	0												
26	GR-24	400	+	0	LR-24	400	++	0												

TABLE II—CONT'D

CASE	POLLEN EXT.	DOSE UNITS	REACTION LCL.	REACTION GENL.	7 DAYS LATER POLLEN EXT.	7 DAYS LATER DOSE UNITS	7 DAYS LATER REACTION LCL.	7 DAYS LATER REACTION GENL.	7 DAYS LATER POLLEN EXT.	7 DAYS LATER DOSE UNITS	7 DAYS LATER REACTION LCL.	7 DAYS LATER REACTION GENL.	7 DAYS LATER POLLEN EXT.	7 DAYS LATER DOSE UNITS	7 DAYS LATER REACTION LCL.	7 DAYS LATER REACTION GENL.
27	GR-24	1000	++	0	LR-24	1000	++++	0	GR-24	2500	++	0	GR-29	2500	+	0	LR-24	2500	++	0
28	GR-24	2500	+++	0	GR-29	2500	0	0	LR-24	2500	+++	0	LR-24	6000	++	0				
29	GR-24	4000	++	0	LR-24	4000	++	0	GR-29	6000	++	0	LR-24	6000	+++	0				
30	GR-24	4000	+	0	LR-24	4000	++++	0	GR-24	6500	++	0	GR-29	7000	++	0	LR-24	7000	+	0
31	GR-24	5000	+++	0	LR-24	5000	+++	0	GR-24	7000	+	0								
32	GR-24	1000	++	0	GR-29	1000	++	0	LR-24	1000	+	0								
33	GR-24	4000	++	0	GR-29	4000	+++	0	LR-24	4000	++	0	GR-29	5000	+++	0	LR-24	5000	++++	0
34	GR-24	2500	++	0	LR-24	2500	+++	0	GR-24	5000	+++	0	LR-24	1500	0	0	LR-32	2000	+++	0
35	GR-24	2000	+	0	GR-29	2000	+	0	LR-24	2000	+	0								
36	GR-29	800	++	0	LR-24	800	++++	++	GR-24	800	+	0	LR-24	800	++++	0				
37	GR-29	1000	+	0	LR-24	1000	+	0	GR-24	2000	+	0	GR-29	2000	+	0	LR-24	2000	++	0
38	GR-29	3000	+++	0	LR-24	3000	+++	0	GR-24	6000	++++	0	GR-29	6500	++	0	LR-24	6500	++	0
39	LR-32	1250	++++	0	GR-29	3000	++	0	GR-29	5000	++	0								
40	LR-32	3500	0	0	GR-29	14000	0	0	LR-32	4000	0	0								
41	LR-32	6000	+	0	GR-29	20000	+	0												
42	LR-32	3500	++++	0	GR-29	10000	++++	+	GR-29	7000	+++	0	LR-32	3500	++++	0				
43	LR-32	3000	+	0	GR-29	9000	0	0	GR-29	15000	0	0	LR-32	3500	+	0				
44	LR-32	5000	++	0	GR-29	13000	++	0	LR-32	5000	++	0								
45	LR-32	3000	+++	0	GR-29	7500	+++	++	LR-32	3000	++	0								
46	LR-32	1750	+	0	GR-29	4000	+	0												
47	LR-32	3000	+++	0	GR-29	7500	+++	0												
48	LR-32	3500	+	0	GR-29	10000	+	0	GR-29	15000	++	0								
49	LR-32	2500	0	0	GR-29	7500	++	+	LR-32	2500	+	0								
50	LR-32	6000	++++	0	GR-29	20000	++++	++	GR-29	15000	+++	0	LR-32	5000	++	0				
51	LR-32	6500	0	0	GR-29	20000	+	0												
52	LR-32	7000	0	0	GR-29	20000	++	0	LR-32	7000	+	0								

0 = no local or general reaction.
+ = amount of local reaction and indicates the severity of the general reaction.

the identical unit dosage of LR-24 or LR-32 was substituted for GR-24 or GR-29 in 37 patients, 15 constitutional reactions resulted. In these 15 patients, when either GR-24 or GR-29 was given at the following visit, no constitutional reactions resulted. A control group of 14 patients treated with LR-32 tolerated an equal dose or one twice as great of GR-24 or GR-29, but a few constitutional reactions resulted with the giant ragweed when its dose was two and a half to three times greater than that tolerated with low ragweed.

These results showed a greater capacity of the low ragweed to produce constitutional reactions. The two giant ragweed extracts diluted on the protein nitrogen unit, although prepared from pollens of different years, were apparently of equal activity as far as local or constitutional reactions could determine. The two low ragweed extracts diluted on the protein nitrogen unit from pollens of different years were alike as far as local or constitutional reactions could determine.

(B) Comparison of Giant and Low Ragweed Extracts Prepared From Pollen Collected in 1933 in New Jersey and California.

While these results had confirmed the previous observation of a greater capacity of the low ragweed to produce constitutional reactions, it was decided to repeat this work, using two giant ragweed pollens and two low ragweed pollens collected in the same year from different sources in order to make sure this property of the low ragweed was a general characteristic of the pollen and not due to mere coincidence or any variation in condition of growth.

Extracts were prepared from pollen collected in the summer of 1933. The giant and low ragweed pollens purchased from Knapp & Knapp were collected in California, while giant and low ragweed pollens were collected by our botanist, Mr. Hodgson, in New Jersey. Four extracts were prepared at the same time from 5 gm. of these ethyl ether extracted pollens in 100 c.c. of alkaline-saline fluid.[14] They were analyzed for total and protein nitrogen and were diluted on the protein nitrogen unit. Table III gives the analyses of these extracts.

TABLE III

RAGWEED EXTRACTS IN ALKALINE SALINE (5 GM. 1933 POLLEN PER 100 C.C.)

POLLEN	SOURCE	SYMBOL	NITROGEN MG. PER C.C.		UNITS PER C.C.
			TOTAL	PROTEIN	
Giant ragweed	F. H.	GR-33B	0.92	0.30	30,000
Low ragweed	F. H.	LR-33B	0.78	0.30	30,000
Giant ragweed	K. & K.	GR-33C	0.96	0.30	30,000
Low ragweed	K. & K.	LR-33C	1.17	0.45	45,000

In October, 1933, 35 new ragweed hay fever cases, none of which had ever been previously treated, were divided into four groups, and inoculations were commenced with the new pollen extracts, one ex-

tract being assigned to each group. Each patient was intracutaneously tested with 10, 100 and 1,000 unit dilutions of all four extracts, and as in the previous study no material differences in the size or activity of the wheals were observed with tests of the same dilutions of the four extracts. Subcutaneous injections were given over a period of six months at weekly intervals. Severe local reactions occurred more frequently in the patients treated with the low ragweed extracts, and it was more difficult to increase the dosage of these groups. The results on changing from the maximal dose of one extract to another are shown in Table IV.

All patients who had received LR-33C took the same dosage of LR-33B, GR-33C or GR-33B. All patients who had received LR-33B took the same dosage of LR-33C, GR-33B or GR-33C. All patients receiving GR-33B took the same dose of GR-33C, and vice versa. In these changes no constitutional reactions resulted, and the local reactions were of similar size.

It was intended to change all the patients receiving giant ragweed extract after six months' treatment to the same dosage of low ragweed. Eleven of 18 patients were changed from giant to low ragweed extract of the same dosage, and four constitutional reactions resulted. In the last case the reaction was so severe that it seemed unwise to continue these changes. In the other seven changed cases more severe local reactions resulted than with the previous dosage of the giant ragweed. All the patients having constitutional reactions were subsequently given equivalent doses of giant ragweed with no difficulty. Two of the patients having constitutional reactions with 10,000 units of low ragweed took 20,000 units of giant ragweed at the next visit with no difficulty. Five patients who had taken 10,000 units of low ragweed were given 20,000 units of giant ragweed at the next visit with no constitutional or severe local reactions.

This work confirmed our previous results. It indicated an identity in the two giant ragweed pollens as well as an identity in the two low ragweed pollens, irrespective of locality or season of growth. It confirmed the greater capacity of the low ragweed to produce constitutional reactions. When a pure species of pollen was used, the protein nitrogen unit was a practical index of strength as far as local or constitutional reactions could determine.

There were three cases which did not fit into the tables but illustrated the importance of the size of the dose given at the time the change was made. These were perennial cases. The following case is cited as a typical example:

Case A was changed on successive weeks from GR-24 1,500 units to GR-29 1,500 units to LR-24 1,500 units with no untoward effect. After continued treatment with GR-33B the patient was changed to LR-33B

Table IV

Local and General Reactions After Changing Therapeutic Injections of Giant and Low Ragweed Extracts at Weekly Intervals

Case	Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.	7 days later Pollen Ext.	Dose Units	Reaction Lcl.	Reaction Genl.
53	GR-33B	10000	0	0	GR-33C	10000	0	0	LR-33B	10000	++	+	LR-33C	10000	+++	++	GR-33B	10000	+	0
54	GR-33B	10000	0	0	GR-33C	10000	0	0	LR-33B	10000	+++	0	LR-33C	10000	++	0	GR-33B	15000	+	0
55	GR-33B	5500	0	0	LR-33B	5500	+++	0	GR-33C	7000	0	0	GR-33B	8500	0	0				
56	GR-33B	10000	0	0	GR-33C	10000	0	0	LR-33B	10000	+	+	LR-33C	10000	+	0	GR-33B	20000	0	0
57	GR-33B	5500	0	0	LR-33B	5500	0	0	GR-33C	7000	0	0	GR-33B	10000	0	0				
58	GR-33B	10000	0	0	GR-33C	10000	0	0	LR-33B	10000	+++	0	LR-33C	10000	++	0	GR-33B	15000	++	0
59	GR-33C	8000	0	0	LR-33C	8000	++	0	GR-33B	8000	++	0								
60	GR-33C	10000	+	0	GR-33B	10000	0	0	LR-33C	10000	++	+	GR-33C	10000	0	0				
61	GR-33C	10000	+++	0	GR-33B	10000	+++	0	LR-33C	10000	++++	0								
62	GR-33C	10000	0	0	LR-33C	10000	+++	0	GR-33B	10000	+	0								
63	GR-33C	10000	+	0	GR-33B	10000	+	0	LR-33C	10000	++++	++++	GR-33C	10000	+	0	GR-33C	10000	+	0
64	GR-33B	10000	+	0	GR-33C	10000	+	0	GR-33B	10000	+	0	GR-33B	12000	+	+				
65	GR-33B	10000	+++	0	GR-33C	10000	+++	0	GR-33B	12000	+++	0								
66	GR-33C	850	++	0	GR-33B	850	++	0	GR-33C	1000	++	0								
67	GR-33C	6500	+++	0	GR-33B	6500	++	0	GR-33C	6500	++	0								
68	GR-33C	10000	+	0	GR-33B	10000	+	0	GR-33B	12000	+	0								
69	GR-33C	10000	++	0	GR-33B	10000	+++	0	GR-33C	10000	++	0	GR-33C	13000	++	0				
70	GR-33C	8000	0	0	GR-33B	8000	+	0	GR-33C	10000	+	0								
71	GR-33C	10000	0	0	GR-33B	10000	+	0	GR-33C	10000	0	0								
72	LR-33B	10000	++	0	LR-33C	10000	+	0	GR-33B	20000	+++	0	GR-33C	20000	++	0				
73	LR-33B	8500	+++	0	GR-33C	8500	0	0	LR-33C	8500	+	0	GR-33B	17000	+	0				
74	LR-33B	8000	0	0	LR-33C	8000	0	0	GR-33B	8000	0	0	GR-33C	16000	0	0				
75	LR-33B	10000	+	0	LR-33C	10000	+	0	GR-33B	10000	0	0	GR-33C	10000	0	0				
76	LR-33B	10000	+	0	LR-33C	10000	+	0	GR-33B	10000	0	0	GR-33C	10000	0	0				
77	LR-33B	10000	+	0	LR-33C	10000	+	0	GR-33B	10000	0	0	GR-33C	10000	+	0				
78	LR-33B	2000	++	0	GR-33C	2000	+	0	LR-33C	2000	++	0	GR-33B	5000	+	+				
79	LR-33B	3500	++	0	GR-33C	3500	0	0	LR-33C	3500	++	0	GR-33B	3500	++	0	GR-33B	7000	+++	0
80	LR-33B	8000	++	0	GR-33C	8000	+	0	LR-33C	8000	++	0	GR-33B	10000	++	0				
81	LR-33C	10000	0	0	LR-33B	10000	0	0	GR-33C	10000	0	0	GR-33B	10000	0	0	GR-33C	20000	0	0
82	LR-33C	8500	+++	0	LR-33B	8500	+++	0	GR-33C	8500	+	0	GR-33B	17000	++	0				
83	LR-33C	8000	+++	0	LR-33B	8000	+++	0												
84	LR-33C	2500	+	0	GR-33C	3500	+	0	LR-33B	3500	+	0								
85	LR-33C	5000	++	0	GR-33C	5000	+	0	LR-33B	5000	++	0	GR-33B	10000	++	0	LR-33C	7500	++	+
86	LR-33C	10000	+	0	LR-33B	10000	+	0	GR-33C	10000	0	0	GR-33B	10000	+	0				
87	LR-33C	10000	0	0	GR-33B	10000	0	0	LR-33B	10000	+	0	GR-33C	10000	+	0				

0=no local or general reaction.

+=amount of local reaction and indicates the severity of the general reaction.

at a dosage of 5,500 units. A constitutional reaction occurred in ten minutes. This was moderately severe with rhinitis, conjunctivitis, severe sneezing, nasal obstruction, coughing, and wheezing, and 0.5 c.c. of epinephrine 1:1,000 was given for relief. The local reaction at the site of inoculation was moderate in character. At the next injection GR-33B 5,500 units were given and produced a slight local but no general reaction.

SEROLOGIC STUDY

In this study in addition to the four ragweed extracts (GR-33B, LR-33B, GR-33C, LR-33C) already described, pure water soluble proteins were prepared from the giant and low ragweed (Knapp & Knapp) pollens and used in the comparisons. These proteins were prepared from water extracts of about 700 gm. of ethyl ether extracted pollen of each ragweed, by numerous precipitations with saturation by ammonium sulphate until a water soluble protein solution was obtained. These protein solutions were freed completely from ammonium sulphate by dialysis through hardened membranes, sodium chloride was added to 0.85 per cent, and they were sterilized by Seitz filtration and analyzed[16] for total and protein nitrogen. These protein solutions similar to the ragweed extracts were always diluted according to the protein nitrogen unit. As shown in Table V all the protein nitrogen was precipitated by phosphotungstic acid from these solutions, making total and protein nitrogen the same.

TABLE V

RAGWEED PROTEINS IN PHYSIOLOGIC SALINE

	SYMBOL	NITROGEN MG. PER C.C.		UNITS PER C.C.
		TOTAL	PROTEIN	
Giant ragweed protein	GP-33C	0.50	0.48	48,000
Low ragweed protein	LP-33C	0.44	0.42	42,000

Portions of these protein solutions before the addition of salt were concentrated, and the protein was precipitated by the addition of five times the volume of alcohol. This precipitated protein was then completely dried at 100° C. and analyzed. The analyses in Table V-A showed that the two ragweed proteins were of equal purity.

TABLE V-A

	SYMBOL	ASH AS OXIDE	NITROGEN (ASH-FREE BASIS)
Giant ragweed protein	GP-33C	1.15	13.3%
Low ragweed protein	LP-33C	0.84	13.4%

Phosphorus was absent in both preparations.

Two procedures were resorted to in order to demonstrate if possible any greater activity of low ragweed extract or its protein, that would

TABLE VI.—COMPARISON OF THE REACTIVE CAPACITY OF GIANT AND LOW RAGWEED EXTRACTS BY TESTS OF SITES IN NORMAL DONORS SENSITIZED WITH RAGWEED SENSITIVE SERUMS IN VARYING DILUTIONS

DONOR	SITE	SENSITIVE SERUM	SERUM DILUTION*	RAGWEED EXTRACT 33C	TEST UNITS PER C.C.†	SITE REACTION
M. G.	1	B	1-100	LR	1	+
	2			GR	1	0
	3	B	1-200	LR	10	±
	4			GR	10	±
	5	B	1-300	LR	100	+
	6			GR	100	+
	7	B	1-400	LR	100	+
	8			GR	100	±
	9	S	1-100	LR	1	++
	10			GR	1	+
	11	S	1-200	LR	10	++
	12			GR	10	±
	13	S	1-300	LR	10	++
	14			GR	10	±
	15	S	1-400	LR	10	+
	16			GR	10	0
H. R.	1	B	1-100	LR	1	0
	2			GR	1	0
	3	B	1-200	LR	100	++
	4			GR	100	++
	5	B	1-300	LR	100	++
	6			GR	100	+
	7	B	1-400	LR	100	+
	8			GR	100	0
	9	S	1-100	LR	1	+
	10			GR	1	±
	11	S	1-200	LR	10	++
	12			GR	10	+
	13	S	1-300	LR	10	++
	14			GR	10	+
	15	S	1-400	LR	10	+
	16			GR	10	±
B. G.	1	B	1-100	LR	1	+
	2			GR	1	0
	3	B	1-200	LR	1	0
	4			GR	1	0
	5	B	1-300	LR	10	0
	6			GR	10	0
	7	B	1-400	LR	100	++
	8			GR	100	+
	9	S	1-100	LR	1	++
	10			GR	1	±
	11	S	1-200	LR	1	+
	12			GR	1	±
	13	S	1-300	LR	10	++
	14			GR	10	+
	15	S	1-400	LR	10	++
	16			GR	10	+
A. M.	1	S	1-100	LR	1	+
	2			GR	1	+
	3	S	1-200	LR	1	+
	4			GR	1	±
	5	S	1-300	LR	1	+
	6			GR	1	±
	7	S	1-400	LR	1	+
	8			GR	1	±
	9	S	1-500	LR	1	0
	10			GR	1	0

*0.1 c.c. of these serum dilutions was placed in each site.

†0.025 c.c. test injection.

+ = degree of skin reaction.
± = doubtful skin reaction.
0 = negative skin reaction.

TABLE VII.—COMPARISON OF THE REACTIVE CAPACITY OF GIANT AND LOW RAGWEED PROTEIN BY TESTS OF SITES IN NORMAL DONORS SENSITIZED WITH RAGWEED SENSITIVE SERUMS IN VARYING DILUTIONS

DONOR	SITE	SENSITIVE SERUM	SERUM DILUTION*	RAGWEED PROTEIN 33C	TEST UNITS PER C.C.†	SITE REACTION
	1	B	1- 50	LP	1	+
	2			GP	1	+
O. S.	3	B	1-100	LP	1	+
	4			GP	1	±
	5	B	1-200	LP	10	+
	6			GP	10	±
	1	B	1- 50	LP	1	0
	2			GP	1	0
	3	B	1- 50	LP	10	++
	4			GP	10	+
B. E.	5	B	1-100	LP	10	+
	6			GP	10	±
	7	B	1-100	LP	10	+
	8			GP	10	0
	9	B	1-200	LP	10	±
	10			GP	10	0
	11	B	1-200	LP	100	+
	12			GP	100	0
	1	S	1- 50	LP	0.1	+
	2			GP	0.1	+
	3	S	1-100	LP	10	+++
F. R.	4			GP	10	+++
	5	S	1-200	LP	0.1	0
	6			GP	0.1	0
	7	S	1-200	LP	1	+
	8			GP	1	+
	1	S	1- 50	LP	100	++
	2			GP	100	++
	3	S	1-100	LP	10	0
S. S.	4			GP	10	+
	5	S	1-200	LP	1	0
	6			GP	1	0
	7	S	1-200	LP	10	+
	8			GP	10	±
	1	S	1-200	LP	1	±
	2			GP	1	+
	3	S	1-200	LP	1	±
	4			GP	1	+
	5	S	1-300	LP	1	±
	6			GP	1	+
G. H.	7	S	1-300	LP	1	±
	8			GP	1	+
	9	S	1-400	LP	10	+
	10			GP	10	+
	11	S	1-400	LP	100	++
	12			GP	100	++
	13	S	1-400	LP	1000	+++
	14			GP	1000	+++
	1	S	1-200	LP	1	±
	2			GP	1	±
	3	S	1-200	LP	1	0
	4			GP	1	0
R. K.	5	S	1-400	LP	10	+
	6			GP	10	+
	7	S	1-400	LP	100	++
	8			GP	100	++
	9	S	1-400	LP	1000	+++
	10			GP	1000	+++

*0.1 c.c. of these serum dilutions was placed in each site.

†0.025 c.c. test injection.

+ = degree of skin reaction.
± = doubtful skin reaction.
0 = negative skin reaction.

correspond with or explain its greater capacity to produce constitutional reactions. The first one (A) was to determine whether or not there was a demonstrably greater reactive capacity of the low ragweed in comparison with that of the giant by testing sensitized skin sites in nonsensitive donors, since the direct skin test in sensitive patients had not disclosed any greater activity. The second one (B) was to compare the sensitive-serum-neutralizing capacities of the extracts of the two species of ragweed by mixing them in vitro with sensitive serums before their injection into the skin of nonsensitive donors.

(A) Reactive Capacity of Giant and Low Ragweed Extracts and Proteins.

Preliminary experiments indicated the range of serum dilutions which would sensitize normal skin cells. This varied depending upon the serum as well as the donor.* Ragweed sensitive serums were diluted with physiologic saline as indicated in Tables VI and VII. Two sites were prepared intradermally containing 0.1 c.c. of each serum dilution on the outer surface of the arms or on the backs of these donors. After forty-eight to seventy-two hours when all irritation had subsided, the sites were tested with 0.025 c.c. of giant or low ragweed of the strength indicated. Maximal reactions at the sites within thirty minutes of testing were recorded.

In Table VI are given the reactions produced by varying strengths of GR-33C and LR-33C on sites made in four donors with dilutions of two sensitive serums. The difference in the reactivity was not great. In 21 of the 29 tests on four donors, the low ragweed reaction was recorded as slightly greater.

In Table VII are given the reactions produced by the purified proteins GP-33C and LP-33C when testing the sites made with dilutions of the same two sensitive serums in six donors. There is uniformity in the size of the skin reactions produced. The low ragweed was slightly greater eight times, it was slightly less five times, but it was equal to the giant extract sixteen times.

These slightly greater reactions produced by the low ragweed extract and to a certain degree its protein did not seem to explain the much greater capacity to produce constitutional reactions in sensitive individuals as demonstrated in the clinical test above described. This minimal variation in reactivity was observed only with high dilutions of the ragweeds in high dilutions of sensitive serums in passive transfer sites. The direct skin test on ragweed sensitive individuals and tests of sensitized sites containing undiluted serum were not sufficiently accurate or delicate to detect this variation.

*The term "donor" when used in this paper refers to those non-sensitive individuals who donated normal skin for sites and tests. It does not refer to those ragweed sensitive individuals whose serum was taken for study. These donors gave negative skin reactions to test injections of 0.025 c.c. ragweed extract in 1,000 units per c.c., and were known to be receptive to passive transfer.

TABLE VIII

NEUTRALIZATION OF SENSITIVE SERUM BY GIANT RAGWEED EXTRACT NEUTRALIZED IT AGAINST LOW AS WELL AS GIANT RAGWEED EXTRACT AND VICE VERSA

DONOR	EQUAL VOLUMES* SERUM + RAGWEED EXTRACT 33C		UNITS PER C.C.	SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†	SITE	REACTION TO TEST WITH GR-33C 1000 UNITS PER C.C.†
P. G.	B	GR	5	1	++	2	++
	B	GR	15	3	++	4	+
	B	GR	45	5	+	6	0
	B	Saline		7	++++	8	+++
	B	LR	5	9	±	10	±
	B	LR	15	11	0	12	0
	B	LR	45	13	0	14	±
S. G.	B	GR	5	1	+++	2	++
	B	GR	15	3	++	4	++
	B	GR	45	5	+	6	±
	B	Saline		7	++++	8	+++
	B	LR	5	9	+++	10	++
	B	LR	15	11	+	12	+
	B	LR	45	13	0	14	+
I. G.	B	GR	5	1	++	2	+++
	B	GR	15	3	++	4	++
	B	GR	45	5	0	6	±
	B	Saline		7	++++	8	++++
	B	LR	5	9	++	10	++
	B	LR	15	11	±	12	±
	B	LR	45	13	±	14	±
A. D.	B	GR	50	1	0	2	0
	B	GR	75	3	0	4	0
	B	GR	150	5	0	6	0
	B	Saline		7	+	8	+
	B	LR	50	9	0	10	0
	B	LR	75	11	0	12	0
	B	LR	150	13	0	14	0
R. H.	B	GR	50	1	+	2	0
	B	GR	75	3	0	4	0
	B	GR	150	5	0	6	0
	B	Saline		7	++++	8	+++
	B	LR	50	9	0	10	0
	B	LR	75	11	0	12	0
	B	LR	150	13	0	14	0
S. A.	B	GR	100	1	+	2	±
	B	GR	300	3	0	4	0
	B	GR	900	5	0	6	0
	B	Saline		7	+++	8	+++
	B	LR	100	9	±	10	±
	B	LR	300	11	0	12	0
	B	LR	900	13	0	14	0
B. G.	B	GR	100	1	++	2	+
	B	GR	300	3	+	4	0
	B	GR	900	5	±	6	±
	B	Saline		7	+++	8	+++
	B	LR	100	9	+	10	++
	B	LR	300	11	0	12	0
	B	LR	900	13	0	14	0

*0.1 c.c. of these mixtures of serum and ragweed extract or serum and saline was placed in each site.

†0.025 c.c. test injection.

+ = degree of skin reaction.

± = doubtful skin reaction.

0 = negative skin reaction.

TABLE IX

COMPARISON OF THE SERUM NEUTRALIZING CAPACITY OF GIANT AND LOW RAGWEED EXTRACTS PREPARED FROM POLLENS FROM TWO DIFFERENT SOURCES

DONOR	EQUAL VOLUMES* SERUM	+ RAGWEED	UNITS PER C.C.	SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†	DONOR	EQUAL VOLUMES* SERUM	+ RAGWEED	UNITS PER C.C.	SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	S	GR-33B	10	1	+++		S	GR-33B	225	1	+++
	S	GR-33C	10	2	+++		S	GR-33C	225	2	+++
	S	GR-33B	30	3	+++		S	GR-33B	300	3	++
	S	GR-33C	30	4	+++		S	GR-33C	300	4	++
	S	GR-33B	90	5	+++		S	GR-33B	400	5	++
	S	GR-33C	90	6	+++		S	GR-33C	400	6	++
R. D.	S	Saline		7	++++	C. M.	S	Saline		7	++++
	S	LR-33B	10	8	++		S	LR-33B	75	8	+
	S	LR-33C	10	9	++		S	LR-33C	75	9	+
	S	LR-33B	30	10	+		S	LR-33B	100	10	±
	S	LR-33C	30	11	+		S	LR-33C	100	11	±
	S	LR-33B	90	12	±		S	LR-33B	125	12	±
	S	LR-33C	90	13	±		S	LR-33C	125	13	±

*0.1 c.c. of these mixtures of serum and ragweed or serum and saline was placed in each site.
†0.025 c.c. test injection.
+ = degree of skin reaction.
± = doubtful skin reaction.
0 = negative skin reaction.

TABLE IX—CONT'D

DONOR	EQUAL VOLUMES* SERUM	+ RAGWEED	UNITS PER C.C.	SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	S	GR-33B	10	1	++++
	S	GR-33C	10	2	++++
	S	GR-33B	30	3	++++
	S	GR-33C	30	4	++++
	S	GR-33B	90	5	+++
	S	GR-33C	90	6	++++
A. N.	S	Saline		7	++++
	S	LR-33B	10	8	+++
	S	LR-33C	10	9	+++
	S	LR-33B	30	10	++
	S	LR-33C	30	11	++
	S	LR-33B	90	12	+
	S	LR-33C	90	13	±
	S	GR-33B	225	1	+
	S	GR-33C	225	2	+
	S	GR-33B	300	3	+
	S	GR-33C	300	4	+
	S	GR-33B	400	5	+
	S	GR-33C	400	6	+
A. H.	S	Saline		7	++++
	S	LR-33B	75	8	0
	S	LR-33C	75	9	0
	S	LR-33B	100	10	0
	S	LR-33C	100	11	0
	S	LR-33B	125	12	0
	S	LR-33C	125	13	0

DONOR	EQUAL VOLUMES* SERUM	+ RAGWEED	UNITS PER C.C.	SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	B	GR-33B	300	1	±
	B	GR-33C	300	2	+
	B	GR-33B	400	3	0
	B	GR-33C	400	4	+
	B	GR-33B	500	5	0
	B	GR-33C	500	6	±
R. Y.	B	Saline		7	++++
	B	LR-33B	50	8	++
	B	LR-33C	50	9	+
	B	LR-33B	100	10	±
	B	LR-33C	100	11	+
	B	LR-33B	150	12	0
	B	LR-33C	150	13	0
	B	GR-33B	300	1	+
	B	GR-33C	300	2	+
	B	GR-33B	400	3	0
	B	GR-33C	400	4	±
	B	GR-33B	500	5	0
	B	GR-33C	500	6	0
A. G.	B	Saline		7	++++
	B	LR-33B	50	8	+
	B	LR-33C	50	9	+
	B	LR-33B	100	10	0
	B	LR-33C	100	11	±
	B	LR-33B	150	12	0
	B	LR-33C	150	13	0

(B-1) Neutralization of Sensitive Serum by Ragweed Extracts.

Quantitative neutralization of ragweed sensitive serums was carried out to determine the relative neutralizing capacities of the giant and the low ragweed. Equal volumes of sensitive serums and giant or low ragweed in varying strengths were mixed in sterile tubes. These mixtures were shaken and placed in the ice box for eighteen hours. Intradermal skin sites each containing 0.1 c.c. of such mixtures were then made in the selected donors. Control sites were made containing a mixture of equal parts sensitive serum and saline. This technic was used in preparing the sites studied in Tables VIII to XIII inclusive. The immediate reaction or irritation produced at these sites was noted (see Table XIII), and is discussed in greater detail later. After forty-eight to seventy-two hours when all the reaction or irritation had subsided, the sites were tested with 0.025 c.c. of a 1,000 units per c.c. dilution of ragweed extract. Preliminary experiments, not recorded, were made with many strengths of the ragweeds in the serums under study, and sites were prepared and tested in order to determine the neutralizing range of the two species of ragweed.

It had been shown that neutralization of ragweed-sensitive serum sites by either giant or low ragweed extract[6, 8] or by their purified proteins[13] left the site neutralized or inactive to both extracts. The results in Table VIII in the last four donors confirmed this, since sites neutralized by giant or low ragweed did not react when tested with the other ragweed preparation. In the first three donors, sites partially neutralized with giant ragweed reacted similarly when tested with giant or low ragweed. Sites partially neutralized with low ragweed reacted similarly on test to giant or low ragweed. This is contrary to the findings of Moore, Cromwell and Moore.[9] In the remainder of this paper for the sake of uniformity, sites were tested with the low ragweed 33C.

(B-2) Comparison of the Neutralizing Capacity of the Ragweed Extracts 33B and 33C Prepared From Pollens Collected in New Jersey and California.

Clinically, the two giant ragweed extracts 33B and 33C when diluted on the protein nitrogen unit were identical in activity. Similarly the two low ragweed extracts 33B and 33C were alike. In Table IX are the results of a comparison of the serum neutralizing capacity of the two giant ragweed extracts and the two low ragweed extracts using two sensitive serums in six donors. No difference between the giant ragweeds could be determined. The two low ragweed extracts were also alike. When a pure species of ragweed pollen was used, standardization by the protein nitrogen unit gave extracts of identical strength serologically as well as clinically. The greater neutralizing capacity of the low ragweed was noted in this study.

TABLE X.—SERUM NEUTRALIZING CAPACITY OF GIANT AND LOW RAGWEED EXTRACT

DONOR	EQUAL VOLUMES* SERUM +	RAGWEED EXTRACT 33C	UNITS PER C.C.	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.† SITE		SITE (DUPLICATE)	
Y. F.	B	GR	10	1	+++	2	+++
	B	GR	30	3	+++	4	+++
	B	GR	90	5	±	6	±
	B	Saline		7	++++	8	++++
	B	LR	10	9	+++	10	+++
	B	LR	30	11	0	12	0
	B	LR	90	13	0	14	0
F. R.	B	GR	10	1	+++	2	+++
	B	GR	30	3	+++	4	+++
	B	GR	90	5	±	6	±
	B	Saline		7	++++	8	++++
	B	LR	10	9	+++	10	+++
	B	LR	30	11	0	12	±
	B	LR	90	13	0	14	0
R. H.	B	GR	10	1	+++	2	+++
	B	GR	30	3	++++	4	+++
	B	GR	90	5	±	6	±
	B	Saline		7	++++	8	++++
	B	LR	10	9	+++	10	+++
	B	LR	30	11	+	12	±
	B	LR	90	13	+	14	0
B. G.	K	GR	10	1	++++	2	++++
	K	GR	30	3	++++	4	++++
	K	GR	90	5	+++	6	+++
	K	Saline		7	++++	8	++++
	K	LR	10	9	++++	10	+++
	K	LR	30	11	+	12	++
	K	LR	90	13	+	14	0
G. R.	K	GR	45	1	++++	2	++++
	K	GR	135	3	++	4	++
	K	GR	180	5	+	6	+
	K	Saline		7	++++	8	++++
	K	LR	15	9	++++	10	++++
	K	LR	45	11	++	12	+
	K	LR	135	13	0	14	0
M. A.	K	GR	200	1	++	2	++
	K	GR	250	3	+	4	+
	K	GR	300	5	+	6	+
	K	Saline		7	++++	8	++++
	K	LR	55	9	±	10	±
	K	LR	70	11	0	12	0
	K	LR	85	13	0	14	0
G. H.	S	GR	600	1	+		
	S	GR	1000	2	0		
	S	GR	2000	3	0		
	S	Saline		4	++++		
	S	LR	15	5	++++		
	S	LR	30	6	++		
	S	LR	60	7	0		
R. C.	S	GR	600	1	++		
	S	GR	1000	2	0		
	S	GR	2000	3	0		
	S	Saline		4	++++		
	S	LR	15	5	++++		
	S	LR	30	6	++		
	S	LR	60	7	±		

*0.1 c.c. of these mixtures of serum and ragweed extract or serum and saline was placed in each site.

†0.025 c.c. test injection.

+ = degree of skin reaction.
± = doubtful skin reaction.
0 = negative skin reaction.

Since the ragweed extracts prepared from pollens grown in different localities were alike, and the only determinable variation was between the giant and low ragweed, in the remainder of this paper the work will be confined to the differences between the giant and low ragweed 33C.

(B-3) Serum Neutralizing Capacity of Giant and Low Ragweed Extracts.

In Table IX it is evident that it took much stronger giant ragweed extracts to neutralize the same serum in the same donor than it did low ragweed extracts. In Table X are given the results mostly in duplicate of the quantitative neutralization of three ragweed sensitive serums by giant and low ragweed extracts in eight donors. Serum B was neutralized with an equal volume of 30 units per c.c. LR-33C in two out of three donors tested. The same serum mixed with an equal volume of 90 units per c.c. GR-33C in the same three donors was practically neutralized. Serum B required about three times the concentration giant ragweed as low ragweed for neutralization. Serum K took decidedly stronger extracts for neutralization, but low ragweed 90 units per c.c. always gave the neutral sites while giant ragweed 300 units per c.c. did not completely neutralize sites. Serum S was neutralized by an equal volume of about 60 units per c.c. low ragweed, but between 600 and 1,000 units per c.c. of giant ragweed were necessary for neutralization.

TABLE XI

DONOR	EQUAL VOLUMES*			SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	SERUM + 1-10	RAGWEED EXTRACT 33C	UNITS PER C.C.		
M. A. M.	B	GR	60	1	+
	B	GR	100	2	±
	B	GR	150	3	+
	B	GR	200	4	0
	B	Saline		5	++++
	B	LR	15	6	0
	B	LR	20	7	0
	B	LR	25	8	0
	B	LR	30	9	0
	S	GR	200	10	+++
	S	GR	400	11	++
	S	GR	600	12	±
	S	GR	800	13	0
	S	Saline		14	++++
	S	LR	15	15	++
	S	LR	30	16	+
	S	LR	45	17	0
	S	LR	60	18	0

*0.1 c.c. of these mixtures of a 1:10 serum dilution and ragweed extract or a 1:10 serum dilution and saline was placed in each site.

†0.025 c.c. test injection.
+ = degree of skin reaction.
± = doubtful skin reaction.
0 = negative skin reaction.

The point of neutralization varied particularly with the serums and to a certain degree with the donor; and in order to compare the neutralizing capacity of the two ragweeds on two serums in the same donor, dilutions of the serums were used to avoid too great a concentration of extract or sensitive serums in a donor.

In Table XI are compared two ragweed sensitive serums, B and S, in the same donor. A 1:10 dilution of the serums in physiologic saline was used, and equal volumes of the serum dilutions and extracts were mixed and placed in the donor as formerly described. Under these conditions B serum was neutralized by 15 units per c.c. low ragweed and 200 units per c.c. giant ragweed. The S serum was neutralized by 45 units per c.c. low ragweed and 600 to 800 units per c.c. giant ragweed. These two serums varied, the S serums requiring proportionately stronger extracts for neutralization than the B serum. It was noted that diluted serums required proportionately stronger extracts for neutralization than undiluted serums, but in every instance the low ragweed had the greater neutralizing capacity.

(B-4) Serum Neutralizing Capacity of Giant and Low Ragweed Protein.

A comparison of the neutralizing capacity of the pure proteins GP-33C and LP-33C prepared from giant ragweed and low ragweed was made to determine whether this greater neutralizing capacity of the low ragweed extract was a property of the active protein, or else due to some other factors in the pollen extracts. The results in Table XII showed that this greater serum neutralizing capacity of the low ragweed was a property of the protein. Low ragweed protein in 100 units per c.c. neutralized serums, while it required 4,000 to 6,000 units per c.c. giant ragweed protein to neutralize the same serums. Tests not shown in Table XII showed that sites neutralized with low ragweed protein did not react when tested with giant ragweed and vice versa, confirming the similar results with extracts shown in Table VIII.

These results were additional confirmation of the protein nature of the pollen allergen. Giant and low ragweed proteins, although botanically related and chemically similar, may differ in the proportion of a common active group. Chemical work has indicated differences in the ultimate composition of the two proteins. Further chemical study of these proteins is under way.

(C) Immediate Reactions in Donor's Skin When Passive Transfer Sites Were Prepared.

Coca and Grove[8] and Levine and Coca[17] concluded that the "reagin" (antibody) in sensitive serums was neutralized and deprived of its sensitizing power by admixture with "atopen" (allergen) in vitro.

TABLE XII

SERUM NEUTRALIZING CAPACITY OF GIANT AND LOW RAGWEED PROTEIN

DONOR	EQUAL VOLUMES*			SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	SERUM +	RAGWEED EXTRACT 33C	UNITS PER C.C.		
M. W.	B	GP	400	1	++++
	B	GP	600	2	++++
	B	GP	800	3	+++
	B	GP	1000	4	++
	B	Saline		5	++++
	B	LP	75	6	±
	B	LP	100	7	0
	B	LP	125	8	0
	B	LP	150	9	0
	S	GP	400	10	++++
	S	GP	600	11	++++
	S	GP	800	12	++++
	S	GP	1000	13	++++
	S	Saline		14	++++
	S	LP	75	15	0
	S	LP	100	16	0
	S	LP	125	17	0
	S	LP	150	18	0
M. D. L.	B	GP	400	1	+++
	B	GP	600	2	+++
	B	GP	800	3	+++
	B	GP	1000	4	++
	B	Saline		5	++++
	B	LP	75	6	0
	B	LP	100	7	0
	B	LP	125	8	0
	B	LP	150	9	0
	S	GP	400	10	++++
	S	GP	600	11	++++
	S	GP	800	12	++++
	S	GP	1000	13	++++
	S	Saline		14	++++
	S	LP	75	15	0
	S	LP	100	16	0
	S	LP	125	17	0
	S	LP	150	18	0
R. F.	B	GP	1000	1	+++
	B	GP	2000	2	++
	B	GP	4000	3	+
	B	GP	6000	4	+
	B	Saline		5	++++
	B	LP	100	6	0
	S	GP	1000	7	+++
	S	GP	2000	8	+++
	S	GP	4000	9	++
	S	GP	6000	10	+
	S	Saline		11	++++
	S	LP	100	12	0

Baldwin[18] reported that homologous mixtures in the test tubes produced no larger reactions than controls, but noted many exceptions. Clarke and Gallagher[19] concluded that in order to sensitize skin sites,

TABLE XII—CONT'D

DONOR	EQUAL VOLUMES*			SITE	REACTION TO TEST WITH LR-33C 1000 UNITS PER C.C.†
	SERUM +	RAGWEED EXTRACT 33C	UNITS PER C.C.		
M. V.	B	GP	1000	1	++
	B	GP	2000	2	++++
	B	GP	4000	3	±
	B	GP	6000	4	0
	B	Saline		5	+++
	B	LP	100	6	0
	S	GP	1000	7	+++
	S	GP	2000	8	+++
	S	GP	4000	9	+
	S	GP	6000	10	±
	S	Saline		11	++++
	S	LP	100	12	0

*0.1 c.c. of these mixtures of serum and ragweed extract or serum and saline was placed in each site.

†0.025 c.c. test injection.

+ = degree of skin reaction.

± = doubtful skin reaction.

0 = negative skin reaction.

the "reagin" must have an opportunity to act upon the normal skin cells before coming in contact with the "atopen."

Gay and Chant[20] observed that there were differences between immediate normal skin site reactions produced by "reagin-atopen" mixtures and control serum-saline mixtures, and concluded that neutralization of "reagin" was not complete until after injection into normal tissues. Foran and Lichtenstein[21] concluded that mixtures in vitro of hypersensitive serum with homologous "atopen" reacted upon injection in non-sensitive skin, and that neutralization of the specific properties of "reagin" or "atopen" did not occur in the test tube. Harley[22] concluded that the skin reactions produced by mixtures of sensitive serums and pollen extract in the donor were due to sensitization of the normal skin by the sensitive serums, followed by a reaction produced by the specific pollen extract in the mixture.

In the numerous skin sites prepared during this study with mixtures of sensitive serum and pollen extract and sensitive serum and saline (controls), frequently no definite differences in the activity of the immediate site reactions could be determined. However, it was noted that when there was any variation in the immediate site reactions, the lesser reactions were always given by the control mixtures of sensitive serum and saline. In several cases in certain donors, no immediate reactions were given by the control mixtures of saline and sensitive serums (see Table XIII); although not a single example of a negative site reaction was observed when sites were prepared with mixtures of sensitive serums and the specific pollen extract. In Table

XIII are given examples of these immediate reactions. Sensitive or non-sensitive serums had a certain amount of irritative action in certain donors, and this irritation obscured the reaction of the pollen

TABLE XIII

IMMEDIATE REACTIONS IN DONOR'S SKIN WHEN PASSIVE TRANSFER SITES WERE PREPARED

DONOR	MIXTURE FOR SITES*			IMMEDIATE SKIN REACTIONS WHEN SITE WAS PREPARED WITHIN 30 MINUTES		SITE	REACTION TO RAGWEED EXTRACT TESTED 48 HOURS AFTER SITES WERE MADE†
	SERUM	+ EXTRACT 33C	UNITS PER C.C.	WHEAL	ERYTHEMA		
A. H.	S	GR	400	++	++	1	+
	S	LR	125	++	++	2	0
	S	Saline		±	0	3	++++
	NSS	LR	125	++	++	4	0
	NSS	Saline		++	++	5	0
C. M.	S	GR	400	++	+++	1	++
	S	LR	125	++	+++	2	±
	S	Saline		±	0	3	++++
	NSS	LR	125	++	+++	4	0
	NSS	Saline		++	+++	5	0
R. H.	B	GR	90	++	++	1	±
	B	LR	90	++	++	2	0
	B	Saline		++	++	3	++++
F. R.	B	GR	90	++	++	1	±
	B	LR	90	++	++	2	0
	B	Saline		0	0	3	++++
	B	Saline		0	0	4	++++
V. P.	K	LR	90	++	++	1	0
	K	Saline		++	++	2	++++
J. G.	K	LR	135	++	++	1	0
	K	Saline		+	±	2	++++
G. H.	S	LR	60	++	++	1	0
	S	Saline		+	0	2	++++
	S	Saline		0	0	3	++++
M. W.	B	LP	150	+++	+	1	0
	S	LP	150	+++	++	2	0
	B	Saline		++	0	3	++++
	S	Saline		++	0	4	++++
	NSS	LP	150	++	0	5	0
	NSS	Saline		++	0	6	0
M. D. L.	B	LP	150	+++	++	1	0
	S	LP	150	+++	++	2	0
	B	Saline		++	0	3	++++
	S	Saline		++	0	4	++++
	NSS	LP	150	++	0	5	0

*0.1 c.c. of these mixtures of equal volumes of serum and extract or serum and saline was placed in each site.

†Test injection 0.025 c.c. LR-33C 1,000 units per c.c.

NSS = non-sensitive or normal serum.

+ = extent of skin reaction or erythema or wheal.

± = doubtful reaction.

0 = negative reaction.

extract upon the sensitized cells. These observations are in agreement with the conclusions of Gay and Chant, Foran and Lichtenstein, and Harley. We were unable to confirm Harley's observation that

the pollen extract became bound and inactive when mixed with excess sensitive serums. Ragweed extract mixed with an excess of sensitive ragweed serums and with normal serums gave equally positive skin tests on ragweed sensitive individuals. Our results indicated that no neutralization of either serum antibody or ragweed allergen takes place in vitro, but that the cells of the donor's skin are essential to a reaction which ensues when mixtures of sensitive serum and specific extract are injected together. This may be obscured by a nonspecific reaction to certain serums in certain donors.

SUMMARY

1. Extracts of different lots of giant ragweed pollen, irrespective of year or source, when diluted on the protein nitrogen unit were alike as far as direct skin tests or their capacity to produce constitutional reactions could determine.

2. Extracts of different lots of low ragweed pollen, irrespective of year or source, when diluted on the protein nitrogen unit were alike as far as direct skin tests or their capacity to produce constitutional reactions could determine.

3. Direct intradermal skin tests on ragweed sensitive individuals failed to show determinable differences between the giant and the low ragweed.

4. Low ragweed extracts had a greater capacity to produce constitutional reactions than giant ragweed extracts.

5. Passive transfer sites made with sensitive serum dilutions when tested with giant and low ragweed extracts and their proteins showed slightly greater reactions to the low ragweed.

6. The serum neutralizing capacity of low ragweed extracts and protein was from three to ten times stronger than similar giant ragweed preparations.

7. When a pure species of ragweed pollen was used, the resulting extracts were of equivalent serum neutralizing capacity when standardized on the protein nitrogen unit.

8. Ragweed sensitive serums mixed with ragweed extracts and injected into non-sensitive skin produced an immediate specific reaction.

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STUDIES ON THE ACTIVE SUBSTANCE OF GRASS POLLEN*

I. Activation of a Small Molecular Weight Active Group Through Colloidal Substances

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INTRODUCTION

1. When pollen extract is filtered through a protein-tight celloidin membrane† by means of pressure, positive skin reactions can be obtained with the filtrate in all hay fever subjects, though in a less degree than with the whole extract. The filtrate showing negative protein reactions with very sensitive reagents—i.e., 10 per cent trichloracetic acid, 20 per cent sulphosalicylic acid, Spiegler's reagents, or phosphotungstic acid—demonstrates that the proteins have been completely retained by the membrane.

2. Pepsin as well as trypsin digestion diminishes the action of the pollen extract on the skin; however, it does not cause the reaction to disappear entirely. Even the ultrafiltrate of the digested extract is still active on the hypersensitive skin. The reaction, however, is perceptibly decreased (this skin reacts neither with pepsin nor with trypsin itself).

These two observations, which will be described in detail elsewhere, led us to the supposition that the active agent of pollen extract on the skin is associated with the presence of a small molecular weight substance, for this substance passes through the pores of a protein-tight filter, escapes destruction through proteoclastic enzymes, and after this may even be isolated again by filtration. As the antigen-antibody reactions, to which the specific skin reactions must belong, are considered possible only if the antigen is coupled with heavy molecular weight colloidal substances, while its specificity is supposed to be controlled by certain groups joined to a colloidal nucleus in a definite way, it is evident that we followed the same line of thought with regard to the pollen antigen. We may, therefore, assume that our specific small molecular weight group acts through its linkage to proteins or other colloidal carrier substances present in pollen extract. But as a considerable amount of this small molecular weight group passes through the pores of the celloidin filter, it ought therefore to have been present in the original extract in a free state.

*From the Ear, Nose, and Throat Clinic of the University of Groningen.
†"Ultrafeinfilter" from the Membranfilter-Gesellschaft at Göttingen.

As the result of these considerations and facts, the idea occurred to us to prove the presence of these specific active molecules by coupling them with nonspecific colloidal substances. If this should succeed, the activity of the pollen extract would thus be intensified.

EXPERIMENTS

We had thirteen hay fever subjects, nine of whom underwent nearly all tests, and a number of medical students who submitted themselves for the control tests. All skin tests were made by the intracutaneous method on the skin of the back.

Our pollen extracts of 1 per cent strength were prepared with Coca's alkaline extracting fluid (NaCl pur., 2.5; $NaHCO_3$ pur., 1.25; phenol, 2; aqua destillata sterilis 500) without previous extraction of fatty substances because this appeared to diminish the activity somewhat. The extraction was carried out at room temperature for forty-eight to seventy-two hours.

The substances which were added to the extracts, filtrates, and residues for the study of the activation belonged to different groups, colloidal as well as crystalline. These adjuvants were examined, of course, to ascertain whether or not they themselves caused any positive reaction on the skin, either in the dilutions used in the investigations or in stronger concentrations. As our experimental subjects were of allergic constitution, a certain skin sensitivity to several of the substances was to be expected. If such a substance caused some skin reaction, that particular substance was not used for testing that patient.

The following substances were examined for their activating properties:

Proteins, a. *mixtures*—human serum; b. *isolated animal proteins*—human albumin and globulins* euglobulin (rabbit), globulin (bovine serum), egg albumen; c. *vegetable proteins*—aleuronat, gluten, glutelin and gliadin; *proteids* (complex proteins), casein, hemoglobin; proteinoids, gelatin; *colloidal mixture of unknown composition,* omnadin; *silk peptone; amino acids,* glycine, leucine, tyrosine, tryptophane, sodium glutamine, cystine; *histamine; polysaccharides,* amylum, inulin, glycogen, gum arabic, agar-agar, tapioca; *derivatives of polysaccharides,* dextrin; *monosaccharides,* glucose; *alcohols,* glycerol.

The best way to investigate the activation is to find out first the minimum dilution of the pollen extract to which the skin gives either no reaction at all or only a slight one. For finding this threshold of skin sensitivity, a number of dilutions of the extract or the filtrate was used. They were injected intracutaneously in vertical rows in a dose of 0.02 c.c. each. The dilutions were prepared with the above mentioned extracting fluid of Coca.

*We are indebted to Dr. R. Brinkman, biochemist, at Groningen, for a number of pure substances. The proteins of blood were obtained by him, either by chemical means or by electrodialysis.

It is well known that the skin of hay fever subjects differs considerably in sensitiveness to pollen allergen (compare the reactions of the patients Rof. and P. with those of the others) and that the degree of skin sensitivity is not the same every day. Thereupon, the activator was tested in various strengths; finally the dilutions of the extract-

TABLE I*

ONE PER CENT POLLEN EXTRACT OF FESTUCA RUBRA (RED FESCUE GRASS): ACTIVATOR—HUMAN ALBUMIN (CONCENTRATED)

PATIENT	DILUTION	COMPARATIVE INTRACUTANEOUS REACTIONS				
		ALBUMIN	POLLEN EXTRACT	POLLEN EXTRACT PLUS ALBUMIN	ULTRAFILTRATE OF POLLEN EXTRACT	ULTRAFILTRATE PLUS ALBUMIN
G.	1: 1,000	−	+	++	−	++
	1: 400	−			−	++
	1: 200	−			+	++
	1: 100	−			++	+++
Roe.	1: 1,000	−	−	++		
	1: 100	−	++		−	++
	1: 50	−			±	++
	1: 20	−			++	
Ra.	1: 10,000	−	−	±		
	1: 2,000	−	−	++		
	1: 1,000	−	−	+++		
	1: 200	−	±			
	1: 100	−	++			
Rof.	1:100,000	−	−	±		
	1: 10,000	−	±	++	+	++
	1: 1,000	−	++		++	+++
S.	1: 10,000	−	±	+	−	±
	1: 2,000	−			−	+
	1: 1,000	−	+	++	±	++
	1: 400	−			±	+++
	1: 200	−			+	
	1: 100	−	++			
v/d B.	1: 1,000	−	−	+		
	1: 10	−			−	±
	1: 2	±			±	+
O.	1: 1,000	−	−	+		
	1: 200		+			
P.	1:100,000	−	−	+		
	1: 20,000	−	+	++		
L.	1: 1,000	−	−	±		
	1: 100	−	±	+		
	1: 10		+			
F.	1: 100	−	−	+		
	1: 10		+			

*Corresponding results were obtained with pollen extract of Holcus lanatus (tufted soft grass).

and ultrafiltrate-activator mixtures were injected, and the effiects of these three series were compared. To this end the wheals and erythematous areas were copied on a piece of film placed on the skin fifteen minutes after the injection.

In order to obtain an approximate measure for the degree of activation of the skin reaction, the strength of the pure extract or the ultra-

filtrate corresponding with that of the diluted mixture was determined first every time. Strong extracts are not suitable because the original reaction is already maximal and an increase is hardly perceptible. We thought that we might expect a maximal result by using colloidal proteins as activators. Since these, however, tested beforehand had to be indifferent to the skin of the patient, we made our first experiments with blood serum and albumin from nonallergic individuals.

At the very onset we succeeded in producing a considerable increase in the size of the skin reactions with pollen extract when either one of these two substances was added.

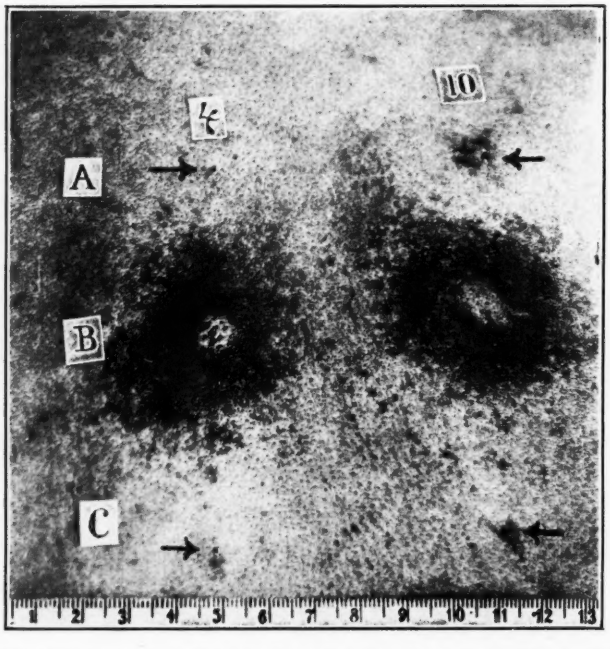

Fig. 1.—Intracutaneous reactions in hay fever patient Ra. The numbers *¼* and *10* indicate the strength of the pollen extract in Noon units (1 unit=0.001 mg. of pollen). In row *A* marked by the arrow are the negative reactions with extract of *Festuca rubra;* in row *C* marked by the arrow are the negative reactions with human albumin of the same concentration as in mixture in row *B;* and in row *B* are the strongly positive reactions with mixture of extract in row *A* and albumin in row *C*.

In order to restrict the tables to the principal data, a number of negative, as well as a number of too strong, reactions have not been recorded. Only those reactions have been recorded which are situated within the range in which the activation can be observed. However, the number of reactions not suited for our purpose grew small as our experience increased. From Table I one may see that the skin reactions with the whole pollen extract, as well as with the ultrafiltrate, are considerably intensified by the addition of human serum albumin.

In most cases the increase in potency was approximately ten times, or 1,000 per cent, in a single case even more (see reactions of patient

Ra.). The positive skin reactions caused by the mixture of pollen extract and albumin in dilutions so extreme that the composing parts separately give a negative result very striking (Figs. 1 and 2).

The experiments with human blood serum as an activator made on the patients Roe. and Rof. with perfectly equal results are not recorded here separately.

After this we tried a few other animal proteins, i.e., bovine serum globulin, and obtained the results given in Table II.

Again the activation of the reaction is ten times or more (patient Roe.). It would take too much space to reproduce all our tables. We will only mention the tests on two patients with euglobulin of rabbit serum mixed with the filtrate of *Festuca rubra* pollen. This time we

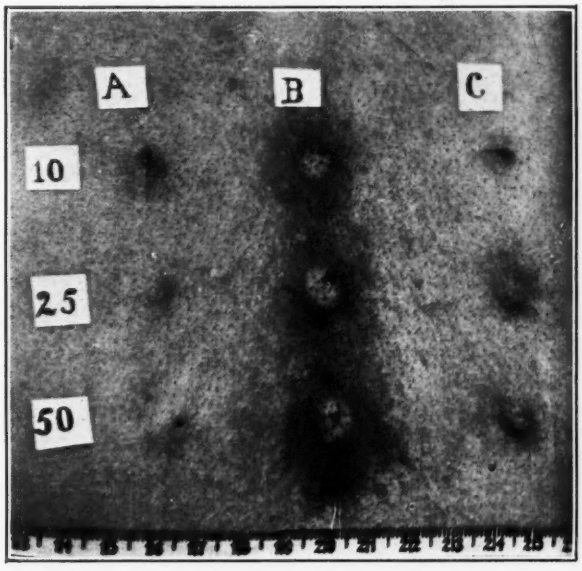

Fig. 2.—Intracutaneous reactions in hay fever patient G. The numbers 10, 25, and 50 indicate in Noon units the strength of the ultrafiltrate used.

In row *A* are the negative reactions with ultrafiltrate of *Festuca rubra;* in row *C* are the negative reactions with human albumin; and in row *B* are the positive reactions with mixture of ultrafiltrate in row *A* and albumin in row *C*.

found in one case an increase of the original reaction and in the other, no difference at all.

The egg albumen, on the other hand, mixed with extract of *Holcus lanatus,* showed in tests on seven hay fever sufferers the same distinct activation obtained with human albumin.

The filtrate of *Festuca rubra* pollen extract was mixed with two different vegetable proteins—aleuronat and gluten. An increase in potency was here also observed, though in a less degree than in the experiments with animal proteins. Of the proteids, we examined casein and hemoglobin, the former mixed with whole pollen extract and the latter with an ultrafiltrate. Tables III and IV show the results.

In Table IV we see again a considerable increase in potency after the addition of a colloidal protein to the original extract and to the ultrafiltrate. Sometimes the reaction is a hundred times stronger, for instance, in the case of patient Roe. after the addition of hemoglobin. As a rule, the increase is approximately ten times.

TABLE II

ULTRAFILTRATE OF 1 PER CENT POLLEN EXTRACT OF DACTYLIS GLOMERATA (ORCHARD GRASS): (ACTIVATOR—BOVINE SERUM GLOBULIN)

PATIENT	DILUTION	COMPARATIVE INTRACUTANEOUS REACTIONS		
		GLOBULIN	ULTRAFILTRATE	ULTRAFILTRATE PLUS GLOBULIN
H.	1:1 000	−	−	−
	1: 100	−	−	+
	1: 10	−	+	+++
	1: 2	−	++	+++
Roe.	1:1,000	−	−	+
	1: 100	−	±	++
	1: 10	±	+	+++
v/d B.	1:1,000	−	−	−
	1: 100	−	−	+
	1: 10	±	±	++
	1: 2		++	

TABLE III

POLLEN EXTRACT USED—(1 PER CENT) OF HOLCUS LANATUS ACTIVATOR—CASEIN

PATIENT	DILUTION	COMPARATIVE SKIN REACTIONS		
		CASEIN	POLLEN EXTRACT	EXTRACT PLUS CASEIN
Roe.	1: 10,000	−	−	±
	1: 1,000		−	++
	1: 10	±		
Ra.	1: 10,000	±	−	+
	1: 1,000	±	+	++
	1: 100		++	
S.	1: 20,000	−	±	±
	1: 10,000	−	±	++
	1: 2,000	−	±	
	1: 1,000	−	++	
Rof.	1:2,000,000		−	±
	1:1,000,000		±	+
	1: 100,000		+	++
	1: 20.000	−	++	++
P.	1: 100,000		−	±
	1: 10,000	−	±	++
	1: 1,000	−	+	++

The experiments with the proteinoid gelatin were of some interest for it was found in the experimental study of anaphylaxis that this substance does not possess antigenic properties—that is to say it is not possible to produce an anaphylactic shock by injecting it in the usual way after a preceding injection. This was ascribed to the fact that gelatin is an incomplete protein, for it lacks the amino acids tyrosine, cystine,

phenylalanine, and tryptophane. When in a certain stage of our experiments we were looking for an immunologic indifferent colloidal carrier substance, our attention was drawn to gelatin. In a trial test the skin of twelve hay fever subjects proved to be insensitive to an intracutaneous injection of gelatin. Two of them reacted faintly on a rather high dilution. The results of our tests are stated in Table V.

TABLE IV

ULTRAFILTRATE OF POLLEN EXTRACT OF DACTYLIS GLOMERATA (1 PER CENT): ACTIVATOR—HEMOGLOBIN

PATIENT	DILUTION	COMPARATIVE SKIN REACTIONS		
		HEMOGLOBIN	ULTRAFILTRATE	ULTRAFILTRATE PLUS HEMOGLOBIN
H.	1: 100	−	−	+
	1: 10	−	+	++
Roe.	1:1,000	−	−	+
	1: 100	−	±	++
	1: 10	±	+	+++
v/d B.	1: 100	−	−	+
	1: 10	±	±	++
	1: 2		++	

TABLE V

ONE PER CENT POLLEN EXTRACT OF HOLCUS LANATUS AND OF FESTUCA RUBRA USED ALTERNATELY: ACTIVATOR—GELATIN (ONE PER CENT)

PATIENT	DILUTION	COMPARATIVE INTRACUTANEOUS REACTIONS		
		GELATIN	POLLEN EXTRACT	EXTRACT PLUS GELATIN
Roe.	1: 10,000	−	±	+
	1: 1,000	−	−	++
C v/d B.	1: 2,000	−	−	±
	1: 1,000	−	−	+
Ra.	1: 2,000	−	−	++
	1: 1,000	−	+	+++
S.	1: 10,000	−	±	++
Rof.	1:10,000,000	−	−	−
	1: 1,000,000	−	±	+
	1: 100,000	−	+	++
H.	1: 1,000	−	±	+
	1: 100	±	+	++
L.	1: 1,000	−	−	−
	1: 100	−	−	±
	1: 10	−	±	+
P.	1: 1,000,000	−	−	−
	1: 100,000	−	−	±
	1: 10,000	−	+	++
F v/d B.	1: 10,000	−	−	−
	1: 2,000	−	−	±
	1: 1,000	−	±	++
	1: 100	−	++	
X	1: 2,000	−	−	±
	1: 1,000	−	±	+
	1: 100	−	+	

The increase in potency noted in this experiment, which proved to be of the same order as the increases of the preceding experiments, will be of great value in the further study of this phenomenon.

When we consider our results with the proteins, we may conclude that their potentizing action is constant. The whole extract as well as the ultrafiltrate has been rendered more active. (Only once we obtained a negative result with rabbit serum euglobulin. We had to ascribe this afterward to some technical error.)

In continuing our series of experiments we come to omnadin, a colloidal mixture, the exact formula of which is unknown to us; it is said to contain a colloidal protein. With this compound we obtained the same results in patients Roe. and Rof. as with the previously mentioned substances.

After this we investigated the derivates of the proteins on their activating properties. The first to be examined was silk peptone, then some six amino acids and histamine. With the silk peptone we saw only twice a distinct activation in five tested individuals. There are two possible explanations: the first, that the rather large peptone molecule acts like a protein, though more moderately; the second, that the commercial preparation which we used contained a number of unaltered protein particles.

We got the following results with the amino acids: tryptophane, no activation in six patients; tyrosine, no activation in six patients; sodium glutamine, no activation in seven patients; leucine, no activation in two and activation in four patients; glycine, activation in five patients; cystine, no activation in one and activation in three patients. Later on we shall explain these positive reactions from the amino acids.

We could use only weak dilutions (10^{-6} to 10^{-8}) of histamine as the skin would otherwise have reacted to this substance itself. In five individuals we obtained entirely negative results, and in one, a distinct activation.

Now we will turn our attention to the group of carbohydrates.

As polysaccharides are considered by a number of investigators to possess antigenic properties, it was of special interest to us to know whether they can act as a colloidal carrier substance for the specific group of pollen extracts. We can summarize the results of the polysaccharides as follows: amylum produced no activation of the pollen extract in four hay fever subjects; inulin, no activation of the pollen extract in five patients; glycogen, no activation of the pollen extract in two patients and activation in three; gum arabic, no activation of the pollen extract in two patients and activation in four; agar agar, no activation of the pollen extract in four patients and slight activation in one.

If we exclude gum arabic, which may contain traces of vegetable protein, only glycogen remains; this, as an animal colloidal poly-

saccharide, may cause activation although less constantly than the proteins do. It is possible that our commercial preparation also contains traces of protein.

With reference to a remark of Caulfeild[16] that the Connaught Laboratory mixes diphtheria toxin with tapioca, in order to acquire a higher antibody production in their horses, it seemed important to us to test also this substance, which consists mainly of polysaccharides. In fact, tapioca proved to have a strong activating effect on two hay fever subjects, though the substance itself acted indifferently on the skin. It is possible that traces of proteins present in this flour have formed the component carrier substance and that the activation of toxin is a phenomenon analogous to ours, though we could not prove the presence of protein by a number of sensitive protein reactions.

Dextrin, a derivate of the polysaccharides, produced no activation of the pollen extract in six patients. Of the monosaccharides, glucose produced no activation of the pollen extract in four patients; and of the alcohols, glycerol produced no activation of the pollen extract in five patients; and one activation of the pollen extract in two patients. Summarizing our observations, we come to the conclusion that the skin reactions with the whole pollen extract as well as with the ultrafiltrate can distinctly be intensified. This activation is a constant one when proteins and the proteinoid gelatin are used as an activator. The results obtained with all the other substances with the exception of glycocoll are negative or less constant. These experiments give reason for the supposition that the specific activity of the pollen extract on the skin of hay fever patients is attached to a small weight molecular substance, which passes through protein-tight filters and needs for its activity some larger complex. These considerations are in agreement with the modern conception of different biochemical processes.

According to Willstätter,[1] the action of the enzymes is to be attributed to a small, specifically active group which attaches itself to a colloidal carrier substance (protein), attaching its activity by means of this linkage only. Digestion, or heating, the protein renders the enzyme inactive. Schulman and Rideal[2] succeeded in reactivating such an inactive enzyme (trypsin) by means of spreading it together with its substrate. Another analogy is to be found in Landsteiner's haptenes.[3] Here again a small molecular group (azosubstance) becomes active only after attaching itself to a larger colloidal carrier substance. The serologic specificity of the new complex is not dependent on the carrier substance, but on the smaller group introduced into it. In order to establish a reaction between antibody and antigen, it is not even necessary to combine the azosubstance to a heavy molecular and colloidal carrier component. The so-called precipitin-inhibition reaction of Landsteiner and Halban, which is a specific one, also takes place when the azosub-

stance is attached to peptone and to a number of amino acids. Landsteiner[4] again explained that although the immunizing action of the complex is attached to the protein part of it, the specific action (i.e., the antibody-antigen reaction) is due to a more simply built group.

How does the activation in our case take place, and how is the action of the normal extract and the ultrafiltrate to be explained? In the whole extract as well as in the ultrafiltrate, the small active groups are present in the free state. If the activator is added, these free groups are bound, either by adsorption or in a more intimate chemical combination. By this linkage they are at the same time protected against the destructive action of elements (as for instance, enzymes) which are present in the tissues and especially in the skin. That is why the large colloidal complexes, like the proteins, are the best suited as "protective colloids," *Schutzkolloiden.* That other substances may afford protection as well is in accordance with the fact that enzymes are protected by glycine, leucylglicine, gum arabic, and glycerol, as was found in the case of invertase by Willstätter and others.

The coincidence of the activation by leucine, glycine, and cystine in our special case, though not a constant one, is remarkable, because they occupy the most important place in the range of amino acids of the skin proteins, while the strongly activating gelatin is also a cutaneous product. The binding takes place almost immediately. In an experiment made to this end at various intervals after the mixing at room temperature, no difference in potency was found between freshly prepared mixtures and those of longer standing. A sufficient quantity of the activator, however, must be added: for example, 1 per cent gelatin to an equal volume of 1 per cent extract; human albumin diluted to plasma concentration to equal parts of extract, etc.

A pure extract brought into the skin may develop its action on the capillaries of the skin in two ways: (a) through the active groups linked to colloids of the pollen, and (b) through the binding of free active groups to the proteins of the skin.

For the ultrafiltrates it is only possible to act in the latter way, and therefore their action must be weaker than that of the whole extract. On the other hand, the residue possessing the action of the group *a* only is stronger than the filtrate but weaker than the whole extract. Our reasoning is based on the hypothesis that an important part of the free, and therefore unprotected, group *b* is destroyed in the skin. How this is brought about by the cells of the skin we are not able to suggest at present. Only if the small groups are protected against the decomposing action of this cutaneous activity by linkage to another body, will they be able to release their activity. We suggest that the so-called activation may be explained in this way. Thus we can understand that the carrier substance of our compound is entirely unspecific and that the

certainty of the result is in direct relation to the size of the protecting molecule. Therefore, the best activation can be obtained by the use of a colloidal protein solution.

An objection to the conception of protective activation through linkage to another molecule might be that the added activator itself would act directly on the skin capillaries and thus intensify the effect of the allergen. Let us suppose that in this case a histamine-like substance is set free; then, if this objection is correct, our activator would also intensify the action of histamine on the skin. We have investigated this point not only for histamine but also for acetylcholine.

Determination of the threshold of activity was carried out on the skin of the backs of four nonallergic students. Afterward a mixture of those substances with gelatin or glycine, which are indifferent to the skin themselves, was injected. In only one case did we see a dubious sensitization to the action of histamine or acetylcholine.

One might argue that the possibility of potentizing ought to be reckoned with, in the sense that substances which are not active in a certain dilution may possibly be active in cooperation. The objection may be made that there are some activators which have no effect on the skin in concentrations which are a thousand times stronger than those used for the activating effect, as for instance, gelatin and human albumin (in a few cases).

Another explanation of the effect of the activators might be that the colloidal carrier substance adsorbs the active groups, thus bringing them in the right position for action. Oppenheimer[5] indicates this for the specific action of the enzymes; a direct proof for this is found in the previously mentioned experiments of Schulman and Rideal. In a later article we will bring forward a few facts which are not in agreement with this explanation.

TABLE VI

COMPARATIVE INTRACUTANEOUS REACTIONS OBTAINED WITH ULTRAFILTRATE OF DACTYLIS GLOMERATA POLLEN EXTRACT

PATIENT	DILUTION	ULTRA-FILTRATE	ULTRA-FILTRATE PLUS 2% GELATIN	ULTRA-FILTRATE OF MIXTURE	RESIDUE OF MIXTURE
G.	1: 1	+	++	−	++
P.	1:1,000	−	−		
	1: 100	+ (weak)	++	−	++
	1: 10	++		±	+++
	1: 1			++	

In order to support our opinion on the phenomenon of activation, we have tested it by the following series of experiments. If it is true that free groups attach themselves to large molecules, such an activated extract or activated ultrafiltrate would more or less lose its activity by

a repeated ultrafiltration, as all groups linked to the large molecules must be retained by the filter. But then the residue left on the filter should be as strongly active as the activated original fluid itself.

As the test is the most accurate with ultrafiltrate of the pollen extract, we mixed it with equal parts of a 1 per cent gelatin solution and subsequently filtered it through a protein-tight membrane. After renewed ultrafiltration of an ultrafiltrate previously highly activated by gelatin, there was indeed a great loss in activity shown in tests on three hay fever subjects. In order to bind still more active groups, we have afterward mixed it with a 2 per cent gelatin solution and obtained an almost complete disappearance of the activity.

From these experiments it may be seen that in the three fluids tested as equal and strong activity is present in the mixture of filtrate and

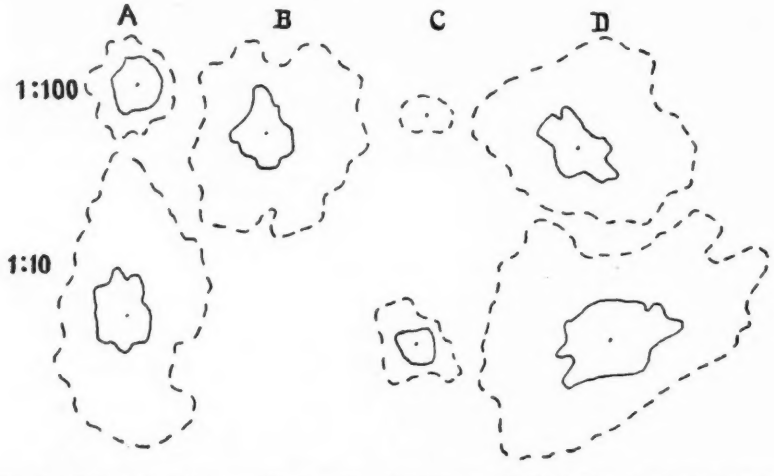

Fig. 3.—Intracutaneous reactions with ultrafiltrate of *Dactylis glomerata* pollen extract. The inner solid line gives the size of the wheal; the outer dotted line, the limit of the erythematous area.

A, ultrafiltrate; *B*, ultrafiltrate plus 2 per cent gelatin (equal parts); *C*, ultrafiltrate of *B*; *D*, residue of the ultrafiltrate of *B*.

gelatin and also in the redissolved residue from the second filtration, whereas the second ultrafiltrate has nearly lost all activity (Fig. 3).

It must be concluded that in accordance with our hypothesis the free groups of the first ultrafiltrate were bound to the added gelatin and so could be retained by a second ultrafiltration.

Investigators are still divided into two camps concerning the active principle of pollen. On one side we find those who are of opinion that the specific agent is no protein. Coca and Grove[6] in 1925 made the first sensational communication, which was afterward confirmed by Walzer and Grove.[7] On the other side we find a number of opponents to this view, L. F. Loeb,[8] the Moores and their collaborators[9] Caulfeild, Cohen, and Eadie,[10] and Stull, Cooke, and Chobot.[11]

When we consider the different experiments, we find that the greater number of the investigators of the second group have precipitated the proteins from the extracts in one way or another and have obtained positive results with this precipitate. This agrees with our conceptions as the proteins have been precipitated and at the same time the linked active groups, while the free groups might be removed from the remaining fluid by adsorption to the precipitate. The strong adsorption of the allergen is evident from the fact that an extract becomes inactive after passing through charcoal or ferric oxide as was found by the two Moores.[12] Even a simple filtration through a Seitz filter decreases the activity of an extract, according to Stull, Cooke, and Tennant.[13]

In L. F. Loeb's digestion experiment with trypsin, dialysis was always performed, and in our conception the liberated active groups were removed with the dialysate, resulting in the end in an inactive residue. It is comprehensible that every digestion must weaken the activity, as the colloidal carriers are destroyed and there remain only the free groups which, as was noted before, have a weaker action.

The results of the dialysis experiments of Moore, Cromwell, and Moore[14] and of Unger, Cromwell, and Moore[15] are in accordance with our experiences.

To start with, the dialysates, consisting of free active groups, gave positive reactions, but in a smaller number than were given by the residues (nondialyzable solutions), which contained the active groups bound to the protein.

It will be the object of further investigations to find whether our conclusions have been right and to explain contradictory facts.

Finally, a word about the possible significance of the above mentioned findings—we cannot answer this question as it is not certain yet whether the substance in the pollen causing the general hypersensitivity, the allergen proper, is the same one which causes the skin reaction, the former being an immunizing mechanism and the latter, an antibody-antigen reaction. In hay fever an absolute parallelism between the patient's condition and his skin reactivity does not always exist. We ourselves know of twenty-two cases of strongly positive skin reactions to pollen without the symptoms of hay fever. This may depend on the individual reactivity—for instance, to local reagin formation—or to the presence of different substances in the pollen.

Through investigations carried on along these lines, we hope to be able to get a better insight into the practical significance of the activation phenomenon.

CONCLUSIONS

1. Our results with ultrafiltration and digestion of pollen extracts led us to the conclusion that the action of pollen extract on the skin is associated with the presence of a small molecular weight substance.

2. The presence of these small molecular weight groups in a free state was demonstrated by binding them to larger substances. Ultrafiltrates as well as whole extracts were rendered more active on the skin when mixed with these larger substances.

3. This activation is supposed to be due to a protective action of the larger complex, which lacks specificity itself. Specificity is only attributed to the small active group.

4. Proteins are the best suited for the purpose of activation (as protective colloids).

5. The conception that a small molecular weight group is bound to a larger carrier substance is in agreement with the modern conception of different biochemical processes which are discussed in the paper.

6. A certain number of well-known facts from the literature on the active principle of pollen have been proved to be in accordance with the experiences described in this paper.

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THE SKIN EXCITING ACTIVITY OF A TIMOTHY POLLEN EXTRACT AS MEASURED BY ITS CONTENT OF THREE NITROGEN FRACTIONS*

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IN A RECENT communication of ours,[1] certain quantitative methods were described for the chemical determination of various nitrogen fractions in solutions of allergenic substances. In one of these, trichloracetic acid was used as the precipitant for protein, and a quantitative analysis of the nitrogen in the precipitate was carried out. Nitrogen values by this technic were compared with the values obtained in the same solution by precipitation of the protein with phosphotungstic acid. In every instance the nitrogen precipitated by trichloracetic acid was less in amount than that thrown down by phosphotungstic acid. In the discussion it was pointed out that this difference was due to the fact that trichloracetic acid is a more specific precipitant for true protein than is phosphotungstic acid, as the latter carries down other nitrogen-containing substances as well as protein. In our conclusions we suggested that trichloracetic acid might prove of value in the chemical standardization of solutions of substances whose allergically active principle is known to be a true protein.

Spain and Newell,[2] by ultrafiltration through cellophane, removed the allergically active substance from ragweed extracts almost completely, but reduced the nonprotein nitrogen concentration only slightly. Several workers[3, 4] have shown that protein is removed from allergen solutions also by passage through bacterial filters, while the nonprotein nitrogen decreases but little under this treatment. By repeatedly passing a timothy pollen extract through Seitz filters, we demonstrated[1] that all nitrogen fractions were decreased although in different proportions. The percentage loss was greater in the nitrogen precipitated by trichloracetic acid than in the nitrogen precipitated by phosphotungstic acid, and least in the total nitrogen. The repeatedly filtered extract had undergone only a quantitative change, since there is no evidence that filtration alters such a solution qualitatively. Therefore, a method was at hand for estimating the relative accuracy of each of the nitrogen fractions as a measure of allergic activity, by comparing the repeatedly filtered extract with the original extract by means of skin tests.

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METHODS

A 3 per cent extract of defatted timothy pollen was prepared in Coca's solution and filtered once through a Seitz filter to secure sterility. A portion of this solution was subjected to eight further passages through Seitz filters. Chemical determinations of total nitrogen (TN), phosphotungstic acid precipitable nitrogen (PN pta), and trichloracetic acid precipitable nitrogen (PN trichl) were carried out on both original and repeatedly filtered portions. The original extract was labelled TS, and the repeatedly filtered portion, TS9. The nitrogen values found in both preparations are given in Table I.

TABLE I

NITROGEN CONTENT OF FILTERED AND UNFILTERED EXTRACTS OF TIMOTHY POLLEN

EXTRACT	TOTAL NITROGEN	NITROGEN* PRECIPITATED BY PHOSPHOTUNGSTIC ACID	NITROGEN† PRECIPITATED BY TRICHLORACETIC ACID
	mg. per c.c.	mg. per c.c.	mg. per c.c.
Unfiltered TS	0.429	0.290	0.160
Filtered TS9	0.267	0.109	0.035

*Nitrogen precipitated by phosphotungstic acid was determined by the difference between the total nitrogen and the nonprotein nitrogen after removal of the phosphotungstic acid precipitate by centrifuging.

†Nitrogen precipitated by trichloracetic acid was determined by direct analysis of the precipitated material.

Dilutions in buffered saline solution for skin testing were prepared from each of the two preparations TS and TS9, on the basis of the three nitrogen values found, and are shown in Table II. These strengths were used, since the nitrogen fractions TN, PN pta and PN trichl in the unfiltered extract TS were in the approximate proportions of 1, 0.7 and 0.4 respectively.

TABLE II

TEST DILUTIONS OF UNFILTERED TS AND FILTERED TS9 EXTRACTS

	UNFILTERED TS NITROGEN CONTENT		FILTERED TS9 NITROGEN CONTENT
A*	0.0001 mg. TN per c.c.	B†	0.0001 mg. TN per c.c.
	0.001 mg. TN per c.c.		0.001 mg. TN per c.c.
	0.01 mg. TN per c.c.		0.01 mg. TN per c.c.
A*	0.00007 mg. PN pta per c.c.	C†	0.00007 mg. PN pta per c.c.
	0.0007 mg. PN pta per c.c.		0.0007 mg. PN pta per c.c.
	0.007 mg. PN pta per c.c.		0.007 mg. PN pta per c.c.
A*	0.00004 mg. PN trichl per c.c.	D†	0.00004 mg. PN trichl per c.c.
	0.0004 mg. PN trichl per c.c.		0.0004 mg. PN trichl per c.c.
	0.004 mg. PN trichl per c.c.		0.004 mg. PN trichl per c.c.

"TN" designates total nitrogen; "PN pta" designates nitrogen precipitable by phosphotungstic acid; "PN trichl" designates nitrogen precipitable by trichloracetic acid.

*These three groups represent only a single solution, serially diluted, of the unfiltered extract, the figures indicating simply the proportions of the three nitrogen fractions therein.

†Three separate solutions, each diluted serially, were required to prepare these dilutions, since the three nitrogen fractions were present in the filtered extract in different proportions than in the unfiltered extract.

The first, or strongest dilution, was made by adding to a rubber stoppered vial containing a known volume of sterile buffered saline solution the calculated amount of the undiluted pollen extract so as to make the final volume contain the desired amount of nitrogen per cubic centimeter. This was then diluted serially to make the further dilutions.

The details as to the volume of undiluted extract, and the content of the three nitrogen fractions, in the initial or strongest dilutions of each of the two extracts are given in Table III.

TABLE III

COMPOSITION OF FIRST DILUTIONS OF UNFILTERED AND FILTERED EXTRACTS

EXTRACT	VOLUME OF CONCENTRATED EXTRACT IN 1 C.C. OF DILUTION	TOTAL NITROGEN	NITROGEN PRECIPITATED BY PHOSPHOTUNGSTIC ACID	NITROGEN PRECIPITATED BY TRICHLORACETIC ACID
	c.c.	mg. per c.c.	mg. per c.c.	mg. per c.c.
TS	A† 0.024	0.01*	0.007*	0.004*
TS9	B† 0.037	0.01*	0.004	0.0013
TS9	C† 0.064	0.018	0.007*	0.0022
TS9	D† 0.115	0.034	0.012	0.004*

*The figures with asterisks indicate, for each dilution, the content of the nitrogen fraction on which the skin tests were based.

†For explanation of these letters, refer to Table II.

It is evident that each of the three initial dilutions of TS9, containing respectively 0.01 mg. per c.c. of total nitrogen, 0.007 mg. per c.c. of nitrogen precipitable by phosphotungstic acid, and 0.004 mg. per c.c. of nitrogen precipitable by trichloracetic acid, was equivalent in respect to one nitrogen fraction to the first dilution prepared from the unfiltered preparation TS; in other ways, however, each was dissimilar. TSA and TS9B (Table III), for example, contained the same concentration of total nitrogen, but their content of nitrogen precipitable by phosphotungstic acid and trichloracetic acid was very different.

COMPARATIVE SKIN TESTS

The patients chosen for the skin tests were proved cases of grass hay fever, and were classified as to skin sensitivity according to the method of Cooke:[5]*

CASE 1.—F. W., female, aged nineteen years. Class I (active). This patient was a constitutional reactor. Generalized urticaria and slight asthma occurred several times when the therapeutic doses were increased by small amounts. Skin reactions to the comparative tests were active and spreading. Maximum therapeutic dose reached, 1,000 p. u. Relief "95 per cent."

*Class I gave a marked reaction to a solution containing 10 protein units per c.c., Class II a marked reaction to a solution of 100 p. u. per c.c., and Class III a marked reaction to a solution of 1,000 p. u. per c.c.

CASE 2.—A. W., female, aged twenty-one years. Class I. Skin reactions to comparative tests clear cut. Maximum dose reached, 3,000 p. u. Relief "90 per cent." Also sensitive to ragweed.

CASE 3.—C. W., male, aged eleven years. Class I-II. Skin reactions to comparative tests clear cut. Maximum dose reached, 7,000 p. u. Relief "75 per cent." Perennial mild asthma; also sensitive to ragweed, orris root and certain foods; bacterial allergy present.

TABLE IV

COMPARATIVE SKIN TESTS

PATIENT	UNFILTERED TS MG. PER C.C.	COMPARATIVE SIZE OF SKIN REACTIONS	FILTERED TS9 MG. PER C.C.
	(A) Dilutions Based on Content of Total Nitrogen		
F.W.*	0.0001 TN	=	0.0001 TN
	0.001 TN	=	0.001 TN
	0.01 TN	=	0.01 TN
A. W.	0.0001 TN	=	0.0001 TN
	0.001 TN	>	0.001 TN
	0.01 TN	>	0.01 TN
C. W.	0.0001 TN	>	0.0001 TN
	0.001 TN	>	0.001 TN
	0.01 TN	>	0.01 TN
C. M. S.*	0.0001 TN	=	0.0001 TN
	0.001 TN	>	0.001 TN
C. J.	0.001 TN	>	0.001 TN
	0.01 TN	>	0.01 TN
D. K.	0.01 TN	>	0.01 TN
	(B) Dilutions Based on Content of Nitrogen Precipitable by Phosphotungstic Acid		
F. W.*	0.00007 PN pta	>	0.00007 PN pta
	0.0007 PN pta	>	0.0007 PN pta
	0.007 PN pta	>	0.007 PN pta
A. W.	0.00007 PN pta	=	0.00007 PN pta
	0.0007 PN pta	>	0.0007 PN pta
	0.007 PN pta	>	0.007 PN pta
C. W.	0.00007 PN pta	=	0.00007 PN pta
	0.0007 PN pta	>	0.0007 PN pta
	0.007 PN pta	=	0.007 PN pta
C. M. S.*	0.00007 PN pta	>	0.00007 PN pta
	0.0007 PN pta	>	0.0007 PN pta
C. J.	0.0007 PN pta	>	0.0007 PN pta
	0.007 PN pta	>	0.007 PN pta
D. K.	0.007 PN pta	>	0.007 PN pta
	(C) Dilutions Based on Content of Nitrogen Precipitable by Trichloracetic Acid		
F. W.*	0.00004 PN trichl	<	0.00004 PN trichl
	0.0004 PN trichl	>	0.0004 PN trichl
	0.004 PN trichl	>	0.004 PN trichl
A. W.	0.00004 PN trichl	=	0.00004 PN trichl
	0.0004 PN trichl	=	0.0004 PN trichl
	0.004 PN trichl	=	0.004 PN trichl
C. W.	0.00004 PN trichl	=	0.00004 PN trichl
	0.0004 PN trichl	=	0.0004 PN trichl
	0.004 PN trichl	=	0.004 PN trichl
C. M. S.*	0.00004 PN trichl	=	0.00004 PN trichl
	0.0004 PN trichl	<	0.0004 PN trichl
C. J.	0.0004 PN trichl	=	0.0004 PN trichl
	0.004 PN trichl	>	0.004 PN trichl
D. K.	0.004 PN trichl	=	0.004 PN trichl

*These were highly sensitive patients with rather spreading skin reactions.

CASE 4.—C. M. S., female, aged thirty-six years. Class I. Mild constitutional reactions on two occasions. Skin reactions to comparative tests rather active and spreading. Maximum dose reached, 3,500 p. u. Relief "100 per cent." Also sensitive to ragweed.

CASE 5.—C. J., female, aged eleven years. Class II-III. Skin reactions fairly clear cut. Maximum dose reached, 5,000 p. u. Relief "75 per cent." Also sensitive to ragweed.

CASE 6.—D. K., male, aged fourteen years. Class III. Skin reactions clear cut. Did not complete therapeutic injections. Perennial asthma; sensitive also to ragweed and house dust, and suffering from hypertrophic sinusitis.

The intradermal method of skin testing was used. The injections were made on the anterolateral surfaces of the upper arm. Both arms were used, but corresponding injections of TS and TS9 were always made on the same arm. Care was taken to introduce, as nearly as possible, the same volume of fluid in each injection. Nine injections were made with dilutions of TS and nine with dilutions of TS9. The sites were in parallel vertical rows, corresponding preparations of TS and TS9 being paired horizontally so that they should be on skin of the same thickness, and so that the reactions elicited could be directly compared. The size of the wheal was considered primarily, the flare secondarily, in the comparisons. The skin reactions to the TS dilutions were regarded as controls: the variants were the reactions to the three groups of TS9 dilutions. No attempt was made to record the degree of difference between the skin reactions, but careful note was made as to whether the TS or the TS9 preparation showed the larger reaction. When doubt was entertained as to a difference, the reactions were recorded as equal. A record of all the results is given in Table IV.

DISCUSSION

In the series of tests, considered as a whole, comparisons were made between 42 pairs of similar dilutions. In 40 of these the filtered preparation TS9 produced skin reactions which were *equal to* or *smaller than* corresponding dilutions of the unfiltered TS preparation. By referring to Table III it will be noted that dilutions of the filtered preparation TS9 contained 50 per cent to 380 per cent more of the undiluted extract by volume than did the corresponding dilutions of the unfiltered preparation TS. The TS9 preparation was therefore decidedly weaker allergically than the TS preparation, filtration having removed a considerable amount of the active material.

Taking the dilutions of the two preparations containing equivalent amounts of total nitrogen (TN), there were 14 similar pairs. Of these, the skin reactions to TS9 were smaller in 9 and equal in 5 as compared with reactions elicited by the unfiltered preparation TS. Again referring to Table III, it will be seen that these dilutions of TS9 con-

tained 50 per cent more undiluted extract by volume, 40 per cent less protein by the phosphotungstic acid method, and nearly 70 per cent less protein nitrogen precipitable by trichloracetic acid than did the corresponding dilutions of the unfiltered TS. The active substance was, therefore, less in dilutions of the filtered preparation, although these dilutions contained the same amount of total nitrogen as did the dilutions of the unfiltered preparation.

Considering the dilutions containing equivalent amounts of protein nitrogen as determined by precipitation with phosphotungstic acid (PN pta) there were 14 pairs. In 11 of these the filtered preparation TS9 gave smaller reactions, and in three the reactions were equal to those given by the TS preparation. The TS9 dilutions contained 170 per cent more of the undiluted extract by volume, 80 per cent more total nitrogen and 40 per cent less protein nitrogen precipitable by trichloracetic acid than did the TS dilutions. In spite of this greater content of undiluted extract and of total nitrogen, and equal content of phosphotungstic acid precipitable nitrogen, the filtered preparation TS9 on this basis of dilution was weaker in allergic activity than the unfiltered preparation TS.

There were 14 pairs of solutions containing equivalent amounts of protein nitrogen precipitable by trichloracetic acid (PN trichl). The filtered preparation TS9 gave skin reactions equal to those of the unfiltered TS in 9 pairs, and slightly *larger* reactions in 2 pairs: in 11 of the 14 pairs the filtered TS9 was allergically equal to or stronger than the unfiltered TS. In three pairs it gave smaller reactions. Dilutions of TS9 on this basis contained 380 per cent more undiluted extract by volume, 240 per cent more total nitrogen, and 70 per cent more protein nitrogen precipitable by phosphotungstic acid than did the corresponding dilutions of TS. The correlation of the skin reactions to the two extracts was much closer when compared on the basis of their content of nitrogen precipitable by trichloracetic acid. It would appear, therefore, that in this timothy pollen extract the content of nitrogen precipitable by trichloracetic acid is a better measure of its allergic activity than is the content of total nitrogen or of nitrogen precipitable by phosphotungstic acid.

We are aware of the criticism which can be brought to bear against direct intradermal tests as a means of measuring differences in the skin exciting activity of allergen solutions. Bowman[6] has shown that by direct intradermal tests, an extract containing 25 per cent less nitrogen than a second extract can be shown to be the weaker allergically, but that a difference of 15 per cent could not be demonstrated. The least difference between dilutions of our two extracts was 40 per cent in phosphotungstic acid precipitable nitrogen. Bowman does not give the nitrogen content of her series of dilutions; hence we cannot

strictly compare our method with hers. However, we did not include in our results reactions to a pair of tests when both were negative. When two strengths of paired dilutions elicited maximal skin reactions in a patient, comparison of the stronger pair has been excluded. We did not attempt to estimate the activity of our two extracts by measuring the absolute size of the skin reactions, but considered only the difference in size as between the paired injections. Following this procedure, we found that the dilutions chosen elicited skin reactions in all patients sufficiently graded from maximal to minimal, so that the relative allergic strength of the two extracts was definitely brought out. We believe, therefore, that our method gave an accurate indication of the relative skin exciting activity of the two extracts, within the limits of error established by Bowman.

From the results obtained it appears that repeated filtration of a portion of this timothy pollen extract reduced its allergic (skin exciting) activity when compared with the unfiltered portion by direct skin tests. At the same time the protein nitrogen precipitable by trichloracetic acid was greatly reduced, while the other nitrogen fractions were proportionally less affected. Comparing the unfiltered and repeatedly filtered preparations by skin tests, with dilutions based on their content of three different nitrogen fractions, it was found that the allergic (skin exciting) activity of each preparation paralleled most closely its content of protein nitrogen precipitable with trichloracetic acid.

SUMMARY

An extract of timothy pollen was divided into two portions. One portion was passed repeatedly through Seitz filters to reduce its protein content and allergic activity. Both portions were analyzed chemically for their content of total nitrogen, nitrogen precipitable by the phosphotungstic acid, and nitrogen precipitable by trichloracetic acid. The repeated filtrations reduced all three nitrogen fractions, and the reduction seemed chiefly at the expense of the nitrogen precipitable by trichloracetic acid, which contains all, or nearly all, of the true protein. Parallel dilutions of the two preparations were made on the basis of these three nitrogen values. Comparative intradermal skin tests on timothy sensitive individuals were made with these dilutions in an effort to determine which of the three nitrogen values approached most closely to a measure of the allergic (skin exciting) activity of the pollen extract.

CONCLUSIONS

1. Repeated passage of a timothy pollen solution through a Seitz filter reduces the amount of protein nitrogen in the filtrate.

2. At the same time the allergic (skin exciting) activity of the pollen extract is reduced.

3. The content of nitrogen precipitable by trichloracetic acid in both filtered and unfiltered portions is a better measure of their allergic (skin exciting) activity than is the content of total nitrogen or of nitrogen precipitable by phosphotungstic acid.

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TREATMENT OF RAGWEED HAY FEVER WITH ULTRAFILTERED EXTRACT*

Preliminary Report

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THE object of this study was to determine clinically the value of ultrafiltrated ragweed extract in the treatment of ragweed hay fever patients. In a previous paper[1] there was described a method of ultrafiltration which was employed in an effort to separate by nonchemical means the active protein fraction of a pollen extract from the inactive noncolloidal fraction. It was concluded that by ultrafiltration through a No. 1200 cellophane membrane, the exciting substance could be separated from the nonprotein nitrogen without appreciably diminishing its activity.† A brief résumé of this method is here given.

The standard extract employed in the Hay Fever Clinic of this hospital is composed of equal parts of high and low ragweed saline extracts. A 100 c.c. portion of this (1934) extract (total nitrogen 0.47 mg. per c.c., nonprotein nitrogen 0.28 mg. per c.c., protein nitrogen 0.19 mg. per c.c.) was ultrafiltered through a No. 1200 cellophane membrane, using nitrogen gas at 65 pounds per square inch as a source of pressure. Four days were taken for the filtration to be completed. The dry residue was then redissolved by allowing the cellophane membrane upon which it was deposited to soak overnight in 100 c.c. of buffered saline solution. The residue solution and the filtrate were then passed through Seitz filters. Sterility was confirmed by incubating small portions in broth aerobically and anaerobically over a period of three days. The residue (total nitrogen 0.104 mg. per c.c., nonprotein nitrogen 0.004 mg. per c.c., protein nitrogen 0.10 mg. per c.c.) and the filtrate (total nitrogen 0.32 mg. per c.c., nonprotein nitrogen 0.29 mg. per c.c., protein nitrogen 0.03 mg. per c.c.) were then diluted with the same volumes of fluid necessary to convert the original extract to dilutions of 5, 50, 2,500 and 5,000 units of protein nitrogen (Cooke and Stull) per c.c. Thus the substances separated by the ultrafiltration would be dissolved in concentrations corresponding to those used in each dilution of the original extract.

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†It was also found that the phosphotungstic acid precipitable nitrogen did not seem to correspond exactly with the allergic activity but that its quantitative determination afforded the most practical chemical method yet devised to standardize ragweed pollen extracts. This confirmed the comprehensive studies of Cooke and his coworkers, Vander Veer, Stull, Chobot, Barnard and Tennant. The majority of their articles have appeared in this Journal in the past three years.

In 19 ragweed hay fever cases the comparisons, by the intracutaneous test, of the activity of residue and filtrate dilutions, with the dilutions of the standard extracts as controls, yielded results similar to those previously reported. The residue solutions showed a degree of activity quite comparable with those of the standard dilutions, while the filtrate was active only in its most concentrated or in its slightly diluted (1:5) form. Each patient was tested with the standard ragweed extract and with the residue extract in parallel rows, employing dilutions of 5, 50 and 500 units. All tests were verified by repetition at the following visit. In 5 other cases, treatment was interrupted although a complete recording of tests was made.

In addition to the parallel tests of the active allergenic content of the residue and filtrate, as compared with the control (standard) extract, the contrast in the neutralizing capacity of the residue and filtrate fractions was shown by means of the test tube neutralization procedure. The filtered serums from three highly sensitive ragweed patients were used. Test tube mixtures, placed overnight in the ice box, were made in a proportion of three parts of serum to one part of extract as follows: (1) serum with ragweed residue extract (5,000 units); (2) serum with ragweed filtrate concentrated (0.32 mg. total nitrogen per c.c.); and (3) serum with standard ragweed extract (5,000 units). Separate sites were made in three nonsensitive patients, 0.1 c.c. of each mixture being used. Control sites were made with 0.1 c.c. of each of the three sensitive serums alone.

Upon testing all these sites twenty-four hours later with control ragweed standard extract, 500 units, no activity could be demonstrated in the residue-serum sites (except in one instance where slight activity was evident), thus indicating a high degree of neutralizing activity in the residue extract. In the filtrate-serum sites, upon test, there developed marked positive wheals, showing that this fraction contained very little or no activity or neutralizing power. The controls of standard extract and serum were, of course, negative, and the control of serum alone was positive.

Ordinarily, routine late hay fever treatments in the clinic are given once weekly, beginning about May 15. Because of the delayed start of treatment in this group (June 8 to July 2), these 19 patients were given injections twice a week of the residue extract in ascending amounts, following the routine schedule employed in the clinic for standard extracts. There were six mild constitutional reactions in a total of 377 injections, three of these being in the same patient. This incidence, being somewhat higher than that ordinarily expected,[2] was considered due to the rather intensive schedule of treatment. At the advent of the hay fever season, daily records of the presence and severity of symptoms were required of each patient. The results as compiled from these records compared favorably with those obtained in patients treated in

the Hay Fever Clinic with the standard ragweed extract given according to the usual routine. It may be assumed, therefore, that the immunizing capacity of the extract is not destroyed by the ultrafiltration procedure, the residue fraction, freed from the nonprotein elements, retaining the active allergenic substance. Previously, Stull, Cooke and Chobot[3] had

TABLE I

LIST OF PATIENTS TREATED WITH RESIDUE EXTRACT, SHOWING MAXIMUM DOSES REACHED AND RESULTS OF TREATMENT

CASE NO.	CLASS	NO. OF INJECTIONS	MAXIMUM DOSE	BEGAN TREATMENT	RESULTS OF TREATMENT
1	A	18	Res. U. 650	6/22	Fair
2	C	19	Res. U. 1,500	6/22	Good
3	B	16	Res. U. 1,250	6/29	Fair
4	B	21	Res. U. 1,000	6/29	Good
7	A	20	Res. U. 1,500	7/ 2	Fair
8	B	22	Res. U. 2,500	6/11	Fair
9	B	18	Res. U. 1,500	6/15	Good
10	B	19	Res. U. 1,000	6/11	Good
11	B	19	Res. U. 1,500	6/11	Good
12	C	20	Res. U. 3,000	6/18	Good
14	B	21	Res. U. 1,500	6/18	Fair
15	B	23	Res. U. 2,500	6/28	Excellent
17	C	16	Res. U. 2,500	6/11	Excellent
18	C	24	Res. U. 3,000	6/18	Good
19	B	21	Res. U. 2,000	6/29	Good
20	A	20	Res. U. 1,250	6/11	Good
21	B	23	Res. U. 2,500	6/15	Fair
22	AA	16	Res. U. 100	6/ 8	Fair
24	C	21	Res. U. 3,500	6/11	Good

Injections discontinued Sept. 28, 1934.
Res. U. = Protein units of residue extract.

found that quite satisfactory clinical results could be obtained by treating ragweed hay fever patients with dilutions of the crystalline purified protein, obtained from ragweed pollen.

CONCLUSIONS

From the results obtained in this series of cases, admittedly small, it would seem that upon ultrafiltration of the ragweed pollen extract the immunizing and constitutional producing capacity is retained in the residue fraction. Further study of its use in the treatment of hay fever is being made.

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INTRADERMAL ANTISERUM TESTS: A BACTERIAL-SPECIFIC RESPONSE NOT DEPENDENT UPON SERUM SENSITIZATION BUT OFTEN CONFUSED WITH IT*

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FOR several years I have been testing routinely a rather large number of patients suffering from various infectious diseases with intradermal injections of the antiserums specific for these infections, and prior to the therapeutic intravenous administration of the appropriate antiserum. Control injections of normal animal serums and of antiserums specific for other infections have been made on each patient. The original purpose was the anticipation and prevention of serious primary serum reactions and anaphylaxis in persons who might have been sensitized previously to horse or goat serum proteins.

A close study of the responses to these tests reveals two entirely different kinds of reactions may occur at the test sites. One type of reaction, which is rather well understood as to cause and significance, indicates naturally or artificially acquired sensitization to serum proteins and is properly interpreted as a warning that primary serum reactions or anaphylaxis will occur if intravenous administration of serum from this animal species is carried out in its presence. This reaction consists of urticarial wheals with spreading pseudopodal margins, with surrounding areas of congestion and usually paler central areas, accompanied by itching and smarting and occasionally by constitutional symptoms—malaise, headache, fatigue, chills or chilly sensations—the severity of constitutional symptoms depending upon the degree of sensitivity of the individual. The reaction usually appears promptly, often within a few moments, and is usually well developed in twenty minutes. The import of this reaction is generally appreciated. It will occur in a sensitized individual regardless of whether the provocative injection is made with an antiserum or with a normal serum of the given animal species.

The second type of reaction also occurs at the test site and consists of spreading erythema and slight elevated central edema. The reaction usually appears promptly, within two to four minutes, sometimes less, and rarely not until ten to eleven minutes, and is usually from 3 to 5 cm. in diameter at the end of twenty minutes, sometimes larger. It is usually maximal in from twenty to twenty-five minutes after in-

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jection of the serum, and there is very often an intensification of the reaction after about six hours. Urticaria never occurs at any time, and only very rarely has any of these reactions been accompanied by itching. The erythema, however, is often intense and may spread rapidly. No constitutional symptoms accompany this local reaction. The initial erythema commonly lasts for only a half hour or so and then fades rapidly. This reaction has been seen only when a patient with a given bacterial infection has been skin tested with an antiserum specific for that infection. When individuals exhibit this simple erythematous-edematous reaction to the homologous specific antiserum, control injections of normal serum from the same animal species, and of antiserums specific for other infections, made from the same species, elicit no reactions whatever. Hence this E-E reaction is obviously a bacterial-specific response and wholly unrelated to the phenomena of serum protein sensitization.

In order to clarify the above statement I shall present schematic examples typical of what has occurred in over 500 individuals who have shown this simple E-E reaction. If undiluted antiserums have been used to elicit the response, undiluted normal serums and heterologous antiserums have been used as control injections. Usually all skin tests have been made with serums diluted 1:10.

1. A patient with proved tularemic infection is skin tested with the following antiserums and serums, and the responses are recorded at twenty minutes after intradermal injection:

1. Antitularense goat serum	Erythema 5 × 7 cm.
2. Antitularense horse serum	Erythema 4 × 6 cm.
3. Normal goat serum	No reaction whatever.
4. Normal horse serum	No reaction whatever.
5. Antibrucella goat serum	No reaction whatever.
6. Antistreptococcus horse serum	No reaction whatever.

The patient received 30.0 c.c. of antitularense horse serum intravenously without the slightest sign of untoward reaction.

2. A patient with proved brucella infection. Skin tests and responses recorded as above.

1. Antibrucella goat serum	Erythema 6 × 9 cm.
2. Normal goat serum	No reaction whatever.
3. Antitularense goat serum	No reaction whatever.
4. Antistreptococcus goat serum	No reaction whatever.
5. Antigonococcus goat serum	No reaction whatever.

The patient received 60.0 c.c. of antibrucella goat serum by vein without any sign of untoward reaction.

3. A patient with proved lymphogranuloma inguinale. Serum skin tests and responses as above.

1. Antilymphogranuloma virus goat serum	Erythema 8 × 13 cm.
2. Antituberculosis goat serum	No reaction whatever.
3. Antitularense goat serum	No reaction whatever.
4. Normal goat serum	No reaction whatever.

The patient received of Tamura's antilymphogranuloma virus goat serum 65.0 c.c. by vein without any sign of untoward reaction.

This E-E reaction is almost constant in tularemic infections. Indeed, it has become a reliable means of quickly confirming the clinical diagnosis. I have records of its presence in all but one of 142 patients, and instances of its occurrence are continually being brought to my attention by physicians who are using antitularense serum. The one patient who failed to give the positive reaction also failed to give a positive skin test response to *B. tularense* standard antigen intradermally. The reaction produced by the specific homologous antiserums is apparently identical in all infections. I have seen it in tularemia, undulant fever, streptococcus infections, gonococcus infections, lymphogranuloma inguinale, tuberculosis, staphylococcus infections, and typhoid fever. It is not constantly observed in streptococcus infections or in brucella infections (experience with the other infections is not sufficient to warrant any statements), and this has suggested that the reaction may be not only bacterial specific but species or strain specific. Further observations will be necessary to establish this point, and it is mentioned here only with the hope that others will test it out.

I have given or personally supervised over 200 intravenous administrations of antiserums in the presence of this erythematous-edematous reaction, and have not seen a single primary serum reaction follow, and nothing remotely suggestive of anaphylaxis. My associates have possibly more than doubled this experience in numbers, and with like results. The experience with serum therapy in tularemia alone will account for at least 300 intravenous injections of antitularense serum in the presence of this reaction, and without a single anaphylactic or anaphylaxis-like response. All evidence points clearly toward a bacterial-specific nature of the reaction, and away from a possible serum protein specific response.

This type of reaction has been so frequent in my experience that it seems almost inconceivable it has not been studied and recorded heretofore. If so, the significance of such studies has escaped general recognition, for misinterpretation of the response is exceedingly common and examples of this are continually being brought to my attention. I wish to call (or to recall) general attention to it now for a very practical reason. There are a number of antibacterial serums in cur-

rent use. Few hospitals or physicians keep normal serums on hand for skin test purposes, and the customary procedure is to use a small amount of the appropriate antiserum to skin test each patient for evidences of serum sensitivity before administering the antiserum. These conditions are ideal for the production of the E-E reaction. Unfortunately this reaction is frequently misinterpreted as an indication of serum protein sensitization. This has often led to the abandonment of serum therapy entirely or to the hesitant intramuscular or subcutaneous injection of too small a quantity of antiserum to be of any therapeutic benefit. In either case the patient is deprived of the real value that serum therapy may offer. Indeed, misinterpretation of the reaction has been so frequent that for the past three years our direction sheets for the use of various trial antiserums have carried a paragraph to warn of its occurrence and to forestall probable misunderstandings. Even so, difficulties referable to it continue to arise. The absence of urticaria is usually sufficient to distinguish clearly this reaction from that which occurs in a serum sensitized person's skin.

The E-E reaction described above can be duplicated easily in certain animals, and experiments are being carried out to study its mechanism. These studies will be reported elsewhere.

Aside from the importance of this reaction in relation to antiserum administration it must be clear that it offers a new possibility for prompt and accurate diagnoses in certain infectious diseases. I have pursued this possibility to an extent where its reliability in this respect can be measured with some accuracy only in relation to tularemic infections. In this disease the E-E reaction is apparently highly specific, and equally reliable as the bacterial suspension intradermal test in diagnosis. In every instance where antitularense serums have been used as heterologous serums for skin tests, in nontularemic patients, no reaction has occurred. Further studies on the reliability of the reaction as diagnostic aids in several other infections are being carried out.

As the serum sensitization reaction and the E-E reaction have been confused in the past, I begin to look askance at the generally accepted procedure of desensitization in current use, where often repeated intradermal or subcutaneous injections of antiserum are supposed to desensitize the patient to a degree where antiserum may be given intravenously quite safely. I do not doubt that desensitization is possible by such a procedure, but I strongly suspect that many patients, supposedly sensitized to serum proteins, actually presented intense E-E reactions and were not sensitized to serum proteins, and that the freedom from mishap after intravenous administration of serum was due to this fact and not in any way related to the tedious but comforting procedure of desensitization.

CONCLUSIONS

Antiserum intradermal tests are capable of eliciting two distinctly different reactions. One, the classical serum sensitization response, is widely recognized and generally appreciated in its significance. The other, an erythematous-edematous reaction, characterized by simple erythema with central edema and complete absence of urticaria, is a bacterial-specific response wholly unrelated to serum sensitization. These two reactions are often confused but should not be. The erythematous-edematous reaction is a useful diagnostic aid and may become more so in a number of infectious diseases. An understanding of the nature and significance of this reaction will prevent its misinterpretation as an indication of serum protein sensitization and the consequent withholding of safe, useful antiserums from patients who may need them.

AN ANALYSIS OF THE ALLERGIC FACTOR IN RECURRENT PAROXYSMAL HEADACHES*

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THE conception of some relationship between migraine and what are now termed the allergic diseases is not new. More than seventy-five years ago Trousseau listed migraine with asthma, urticaria, eczema and a rather heterogeneous group of disorders as comprising the exudative diathesis. Recognition that the eating of certain foods occasionally was followed by headache must necessarily have been a very early observation. With the development of the allergic concept the possibility of migraine being an allergic manifestation appears to have been first seriously suggested in 1919 by Pagniez, Vallery-Radot and Nast.[1] First attempts at allergic therapy were devoted chiefly to nonspecific desensitization. Promising results were reported in this field by Miller and Raulston[2] in 1923. In 1927 I[3] reported an attempt to demonstrate conclusively the presence of an allergic factor in migraine.

Of a group of 33 patients showing positive cutaneous sensitization reactions 10 were relieved of their migraine by dietary avoidances based upon the positive reactions, experienced return of symptoms following repeated ingestion of the positive foods, and were again relieved after subsequent continued avoidance.

Confirmation of the conclusions of an allergic factor in migraine appeared shortly, in the work of Rowe,[4] Rowe and Richet,[5] Eyermann,[6] Balyeat and Brittain,[7] Beecher,[8] Rinkel,[9] Goltman,[10] Sweetser,[11] Todd,[12] and others.

In 1933 I[13] presented an analysis of a follow-up of my migraine series after five years' additional study. While in the first report good results from purely allergic treatment had been observed in 36 per cent, in the second series good results were observed in 51 per cent. In 40 per cent the results were excellent, in that the individuals were entirely relieved except on occasions when they by intention or inadvertently partook of prohibited specific foods. Slightly over 10 per cent reported good results in that they were satisfactorily relieved, although they did still have occasional attacks, not attributable to specific foods. These two groups make up the 51 per cent experiencing adequate relief.

There was an additional 17.5 per cent in whom, though the relief was not adequate, the eating of foods to which the individual had been found sensitive by test, did actually cause attacks of sick headache. These per-

*Read by title before the meeting of the Association of American Physicians, Atlantic City, May 2, 1934.

sons still had too many recurrent headaches, but they were further increased in frequency following the eating of specific allergenic foods.

The observation that with allergic therapy alone results had been improved from 36 per cent to 51 per cent in a five-year period and that there was definite evidence of an allergic factor in an additional 17 per cent suggests that with improved therapeutic methods one might anticipate procuring adequate relief, using allergic principles, in from 68 per cent to 70 per cent of so-called migraine.

The present report represents an effort to determine in what way those patients with chronic recurrent or periodic headaches who achieve no relief or insufficient relief differ from those obtaining good or excellent results. Three objectives have been attempted: first, to determine what difference if any exists between the "good result" and the "poor result" groups; second, to find what improvements or changes in the method of study and therapy would be productive of a higher percentage of satisfactory results; and third, to learn more of the incidence, nature, and causes of chronic recurrent or periodic headaches in the population as a whole and in allergic individuals in particular.

PART I: COMPARISON OF GOOD AND POOR RESULTS SERIES

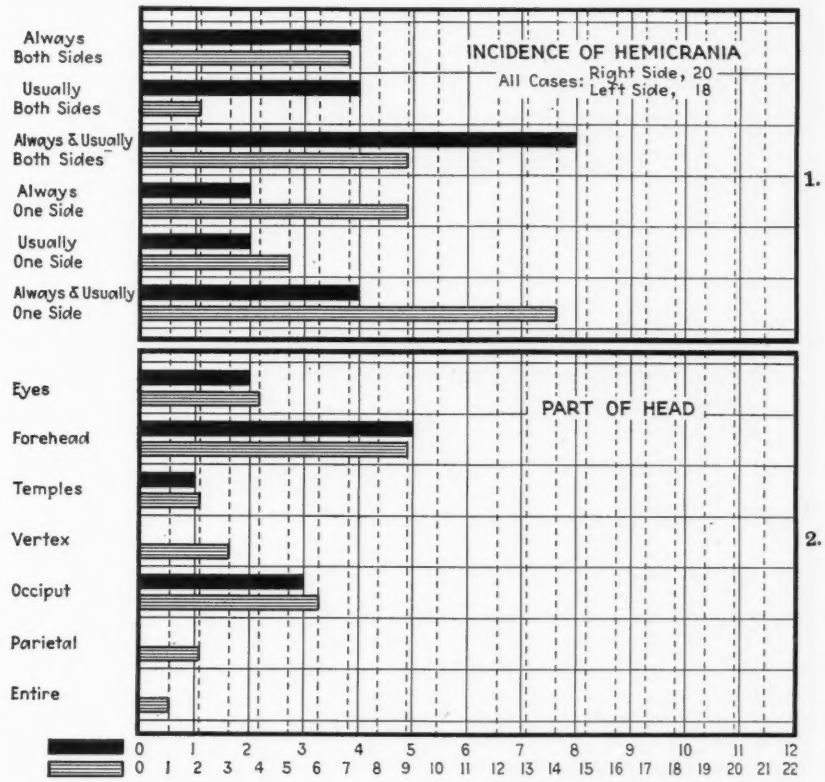
It has been suggested that headaches due to food or other allergens may not be true migraine and should therefore be called by preference "allergic headache." Eyermann and Rinkel in particular have raised this cautionary suggestion. In the following discussion it might be well to recall Osler's definition of migraine, selected because of its simplicity. He defines migraine as "A paroxysmal affection characterized by severe headache, usually unilateral and often associated with vomiting and disorders of vision."

The 1933 report was based upon a study of 63 cases of so-called migraine adequately followed over a period up to eleven years. An attempt was made to analyze this series further. Of these, 37 cooperated in all detail. They form the basis of the following analysis:

Of the 37, 19 reported excellent results in accordance with the criteria mentioned above, 3 reported good results, 3 fair and 12 no relief. In this series, then, 59 per cent obtained satisfactory results. This shows a higher percentage of good results than in the larger series studied eighteen months previously, but the improvement is in the excellent, good and fair series, there being a shift of all toward the excellent side, the percentage with no results remaining the same in the two series. In this analysis unless otherwise stated the good and excellent results series will be grouped together and compared with the no results series, while the fair or borderline cases will not appear in the comparisons.

Incidence of Hemicrania.—By definition, the pain of migraine is usually or often unilateral. It appears from Chart 1 that while some patients with unilateral pain were relieved and others not relieved, de-

cidedly better results were obtained in those in whom the headache was either always or usually on one side. Practically twice as high a percentage of the good results series (shaded columns) had predominantly unilateral headaches as of the poor results series (black columns). Conversely well on toward twice as high a percentage of those not relieved, compared with those relieved, experienced headaches which were usually or always bilateral. It would appear that the probability of good results is somewhat greater when the headache is always or usually unilateral, although this cannot be used as a final



Charts 1 and 2.—Full black represents "poor results" cases out of a total of 12. Broken columns represent "good results" cases out of a total of 22. Comparative length of column may be interpreted as relative percentages.

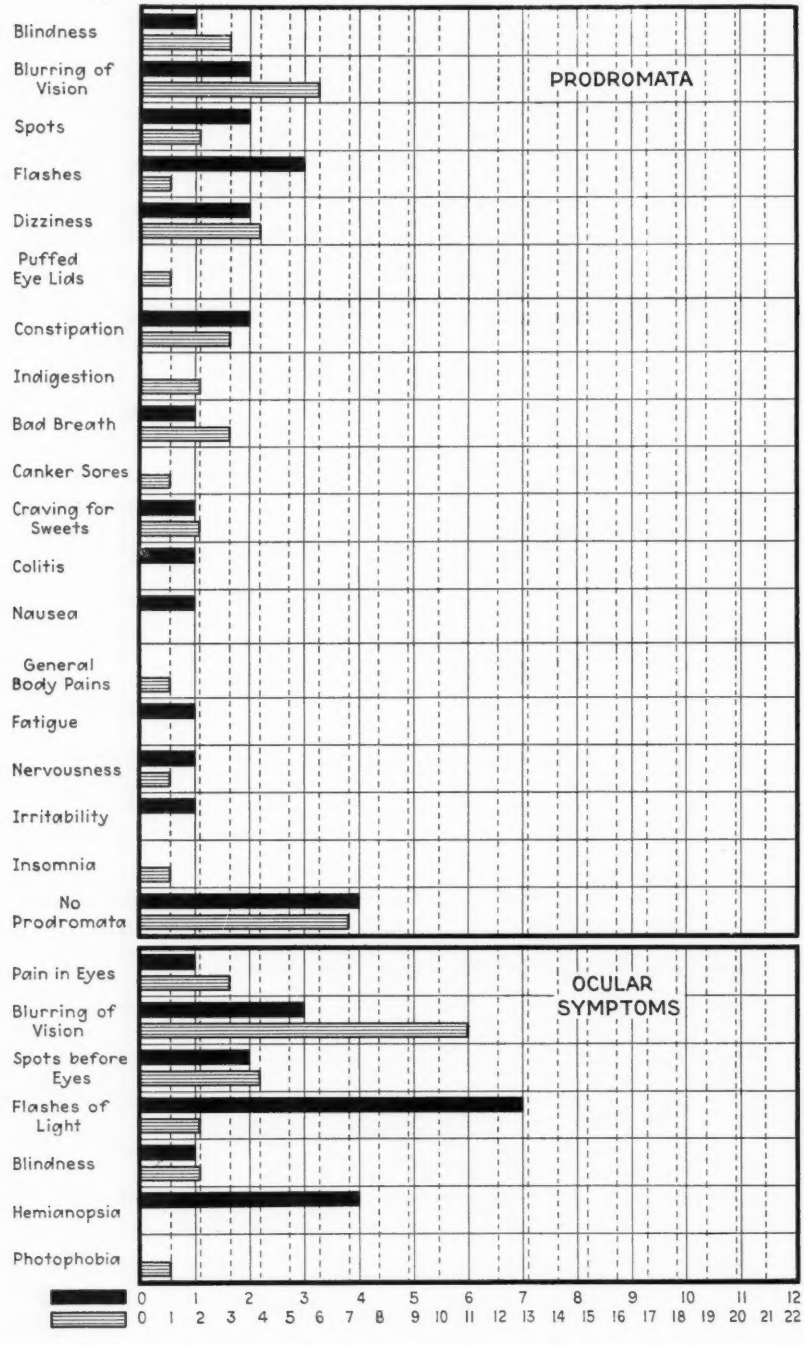
Throughout the charts in this article the black columns indicate poor results; the shaded columns, good results.

criterion inasmuch as excellent results are also observed in individual cases with headache always bilateral, and no relief was obtained in some cases where the pain was always unilateral.

There was practically no variation between the sides predominantly affected; the right side, 20 times; the left, 18.

Location of Pain.—As a rule the pain of the individual attacks commenced in or near the eye. In many cases it spread to the side of the skull and the occipital region. Often it involved one entire half of the

head. Sometimes it began in the occipital region and remained located there. There was no apparent difference between the good and poor re-



3.

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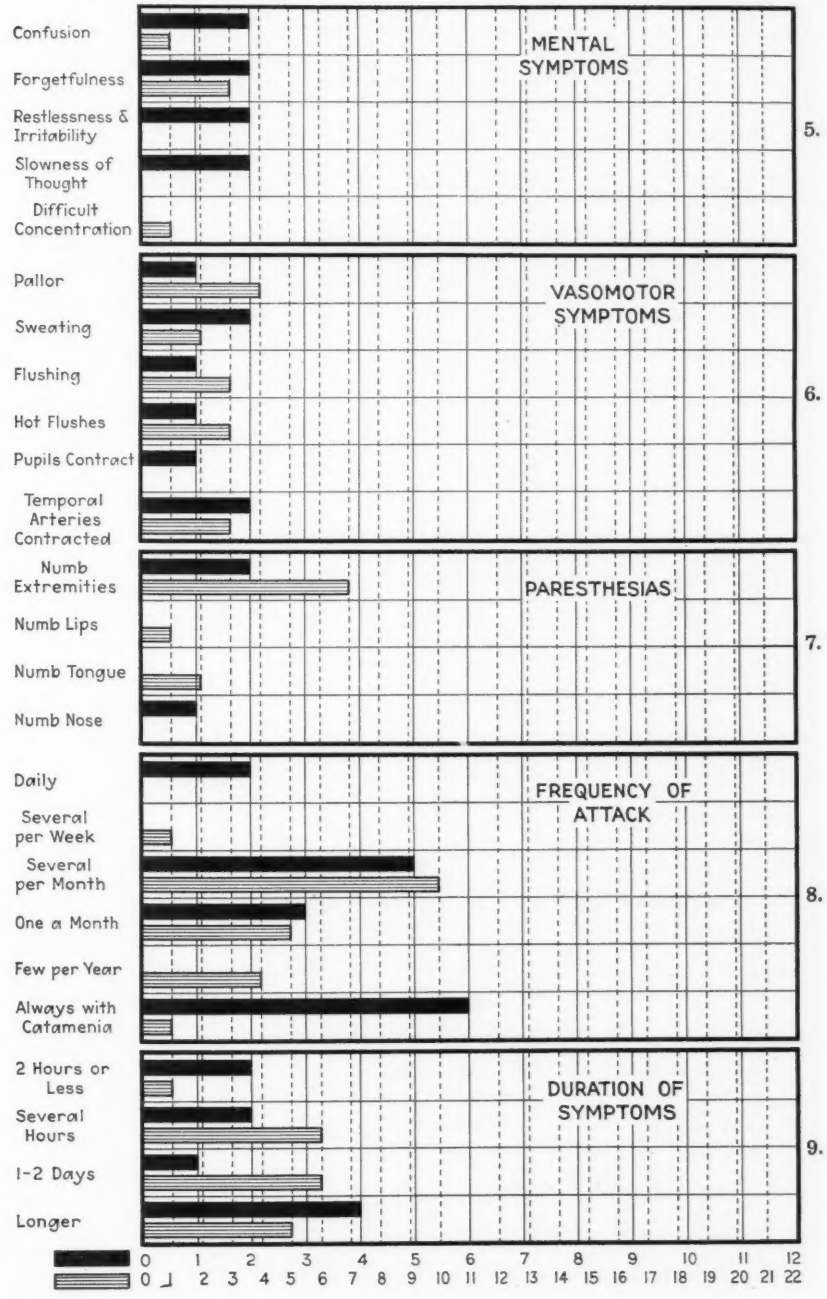
sults series as regards the location of the pain aside from that of unilateral location (Chart 2).

Prodromes.—Of the 37, all but 11 experienced prodromata either occasionally or constantly. The percentage experiencing prodromes was



approximately the same in both good and poor series (Chart 3). The analysis of the prodromes shows no diagnostic difference between the two groups.

Eye Symptoms.—The same is true with regard to ocular manifestations, although it was noted that scotomata and hemianopsia were more frequent in the poor results series (Chart 4).

Mental, Vasomotor and Peripheral Nerve Symptoms.—Charts 5, 6, and 7 show no distinctive difference between the good and poor results series, although it is noted that mental confusion and irritability were slightly more common in the poor results cases, while peripheral paresthesias were slightly more frequent among the good results cases. There was no case of actual paralysis during an attack, although the blurring of vision mentioned by some may have indicated a degree of paresis of the extraocular muscles. Only one patient complained of speech difficulty, due not to paralysis but to swelling of the tongue during the attack.

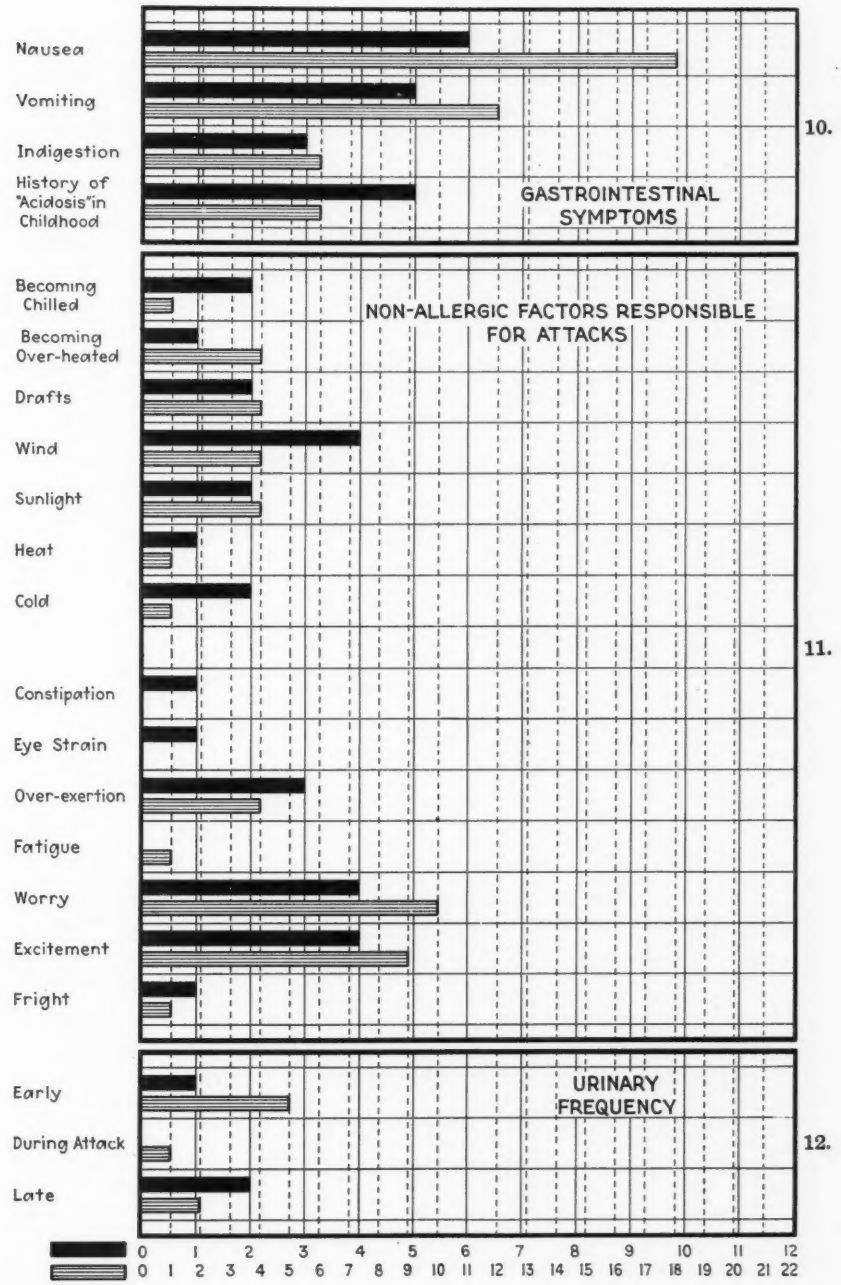
Frequency and Duration.—Chronic daily headaches are more often nonallergic. Headaches appearing regularly with the catamenia are decidedly less likely to respond to purely allergic therapy (Chart 8). The duration of the individual attack gave us no differential diagnostic information (Chart 9).

Gastrointestinal Symptoms.—Here again (Chart 10) we saw no outstanding difference between the two groups. So-called acidosis of childhood, cyclic vomiting, recurrent toxemia, is said to be often the precursor of an adult migraine. Both series gave histories of cyclic vomiting in childhood.

Nonallergic Precipitating Factors.—In my 1933 report it was observed that nonallergic factors, such as fatigue, worry, excitement, constipation, were often responsible for persistence of symptoms. If these nonallergic factors were relatively inactive, attacks due to them were infrequent and the case fell into the good results column. If the nonspecific factors were relatively dominant, headaches would then persist even though a definite allergic factor had been demonstrated. In this event the case fell in the fair or no results series. In the present series nonspecific exciting factors responsible for individual attacks were recorded equally by the good result and the no result cases. (Chart 11.) One might expect *eyestrain* to be recorded more frequently than it appears, but, as a matter of fact, the matter of eyestrain had usually been gone into thoroughly by the patient long before he first reported for allergic study. Of the entire 37, 34 had long since been tested for glasses. Eight had found that glasses gave them some measure of relief.

Urinary Frequency.—Urinary frequency or polyuria has often been mentioned as accompanying attacks of migraine. This is of interest in view of Eyermann's observation[6] that during a migraine attack the individual may gain weight, sometimes as much as five pounds, to be lost rapidly at the subsidence of the attack. Urinary frequency occurred both early and late in both our good and poor results cases,

rather more frequently early, in the good results series. It was a symptom in 36 per cent of the good results and 25 per cent of the poor



results. (Chart 12.) We have no record of weight changes during the attack. A few complained of puffiness of the eyelids and one of swelling of the tongue. Numbness and tingling of the extremities, recorded

under paresthesias, might be associated with some degree of general edema. The possibility of a generalized as well as localized allergic angioneurotic edema should be borne in mind.

Age of Onset and Duration.—In the 1933 series I had observed that the age of onset of migraine corresponded to the usually accepted age range as described by McClure and Huntsinger.[14] Symptoms began before the age of twenty years in 65 per cent and before the age of thirty years in 91 per cent. The duration of the illness appeared to have no effect as regards results. The average duration in those relieved was twenty-one years, in those not relieved, fifteen years. The average duration for the entire series was fifty-two years. The maximum duration in those relieved was fifty-five years, in those not relieved, forty-five years.

Inheritance.—In the present series of 37 cases the percentage of those whose parents had recurrent paroxysmal headaches was the same in the good and bad results. In only one case did both parents suffer therefrom (Chart 13). The good results series shows a definite preponderance of similar type of headaches in brothers, sisters, grandparents, aunts and uncles, but these did also occur in a fair proportion of the poor results. Other allergic manifestations, such as hay fever, asthma, and urticaria, occurred with equal frequency in the immediate families of both groups. The figures are probably incorrectly low because of the ignorance of many patients concerning illnesses in their families. The tentative conclusion may be drawn that the higher the family incidence of similar headaches, the greater the probability of satisfactory results.

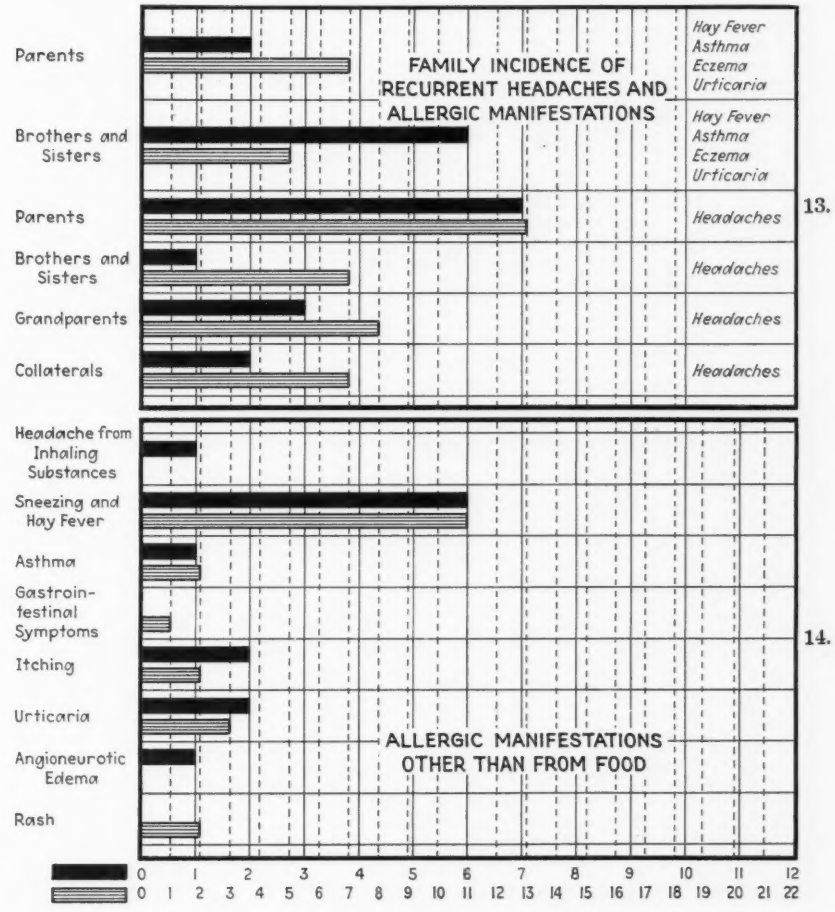
From the foregoing analysis I feel that the conclusion is justified that in both the good and poor results groups, we have been dealing with a series which fulfills the accepted criteria for the diagnosis of true migraine and that the term "allergic migraine" is accurate. It should only be supplanted by the term "allergic headache" in those undoubted cases in which headache of a nonmigrainous type is due to allergy. The migraine being quite typical in both groups there is no way in which we may predetermine the result from the symptomatology, other than to say that in general poor results are obtained when the headaches regularly accompany the catamenia and when they are always bilateral.

Another mode of investigation is available in a study of other allergic manifestations in the individual case.

Other Allergic Manifestations.—Of the 22 good results cases 5 had experienced idiosyncrasy to some foods, an idiosyncrasy causing some allergic manifestation other than headache; 5 were subject to some other allergic manifestation, either nasal, bronchial or cutaneous, not due to foods; and 7 were subject to both food idiosyncrasy and other allergic manifestations. Seventy-seven per cent of the good result

series showed evidence of some allergic manifestation other than migraine. Of the no results series 2 had been subject to an allergic food idiosynerasy, not migraine; 3 were subject to other allergic manifestations and 6 to both. In this unsuccessful series of 12, 91 per cent presented some other allergic manifestations besides headache.

Two criteria have been used in listing these other manifestations of food allergy and allergy involving systems other than the gastrointestinal tract. The first, for example with the foods, has been that the



individual can name the cause, and the cause is a substance which in the majority of normal individuals produces no symptoms or reaction of any nature. The second has been the presence of a symptom commonly recognized as allergic such as asthma, hay fever, repeated explosions of sneezing, urticaria. The observation that the majority of those patients with migraine who obtained no results from allergic therapy do nevertheless present allergic symptoms is most interesting. Symptoms mentioned by those not relieved include angioneurotic edema, urticaria, itching following contact with certain foreign substances,

bronchial asthma, hay fever, and headache following the inhalation of substances such as kerosene smoke and motor exhaust, in concentrations which cause no symptoms in nonallergic individuals. (Chart 14.)

TABLE I

FOODS RESPONSIBLE FOR HEADACHES IN THE SERIES OF 22 WHO OBTAINED RELIEF

Chocolate	7	Peanut	2	Cocoanut	1
Onion	6	Radish	2	Cranberry	1
Wheat	6	Strawberry	2	Cream	1
Cabbage	5	Squash	2	Dill	1
Cucumber	5	Shad	2	Herring	1
Apple	4	Salmon	2	Lamb	1
Peas	4	Wine	2	Leek	1
Pork	4	Buckwheat	2	Mustard	1
Sweet potato	4	Coffee	2	Olive	1
Watermelon	4	Wesson oil	2	Pear	1
Cantaloupe	4	Tuna	2	Pecan	1
Ham	3	Sea foods	2	Peach	1
Peppers	3	Asparagus	1	Perch	1
Pimento	3	Banana	1	Pickles	1
Walnut	3	Beef	1	Raspberry	1
Eggs	3	Brussels sprouts	1	Rutabaga	1
Turnip	3	Blackberry	1	Tea	1
Beans	2	Butternut	1	Tomato	1
Carrot	2	Buttermilk	1	Trout	1
Celery	2	Chicken	1	Veal	1
Garlic	2	Chestnut	1	Corn	1
Lettuce	2	Sardine	1	Spot	1
Mackerel	2	Cauliflower	1	Ginger ale	1
Milk	2	Chicken liver	1	Whisky	1
Olive oil	2				

Figures indicate number of times each food was mentioned.

Foods Causing Symptoms.—The foods responsible for headaches comprise a large group. As is true in other allergic manifestations, almost any substance may be responsible for symptoms in the individual case.

TABLE II

FOODS RESPONSIBLE FOR HEADACHES IN THE SERIES OF 12 WHO FAILED TO OBTAIN RELIEF. ALTHOUGH NOT RELIEVED, THEY FIND A CERTAIN PROPORTION OF HEADACHES TO BE ALLERGIC

Onion	2
Banana	1
Black-eyed pea	1
Chocolate	1
Cocoanut	1
Milk	1
Pickles	1
Pork	1
Sweet potato	1
Salmon	1

The foods found to be responsible for attacks of migraine in the cases relieved (good and excellent in the series) are listed in Table I, together with the number of times each food is incriminated.

Even among those not relieved, certain foods have been recognized as causing symptoms although their avoidance has not benefited the patient. These are listed in Table II.

A large number of foods are listed by the 12 individuals not relieved, as causing other symptoms, which may be allergic. While there might be some question as to the allergic nature of the symptom, there can be no question concerning the fact that in the majority of normal individuals the eating of these particular foods does not produce those symptoms. The foods responsible for food idiosyncrasy among the series of individuals not benefited by allergic therapy are listed in Table III.

It becomes obvious that both the relieved series and the series not relieved suffer from typical migraine, and that both series are equally allergic. If this is true, equally good results should have been obtained by all, providing the nonspecific excitants do not play a predominant rôle. Even so, the probability is that with improved allergic therapeutic methods the percentage of good results may be materially increased. This will be discussed in Part III.

TABLE III

RESPONSIBLE FOODS, AND ALLERGIC SYMPTOMS PRODUCED IN GROUP OF 12, NOT RELIEVED. THESE DEMONSTRATE THE PRESENCE OF AN ALLERGIC FACTOR IN THIS GROUP

Walnut	canker sores	Cabbage	vomiting
Coca cola	colic	Milk	vomiting
Onion	colitis	Orange	vomiting
Peppers	colitis	Coffee	vertigo
Chestnut	heart burn	Peas	urticaria
Cocoanut	heart burn	Beans	urticaria
Gooseberry	heart burn	Apple	indigestion
Pineapple	heart burn	Banana	indigestion
Radish	heart burn	Black-eyed pea	indigestion
Strawberry	heart burn	Cabbage	indigestion
Sweet potato	heart burn	Cheese	indigestion
Cherry	heart burn	Chestnut	indigestion
Cocoanut	nausea	Cocoanut	indigestion
Sweet potato	nausea	Cucumber	indigestion
Shrimp	nausea	Ham	indigestion
Tomato	nausea	Irish potato	indigestion
Walnut	nausea	Lettuce	indigestion
		Mackerel	indigestion
		Peanut	indigestion
		Pickles	indigestion
		Pork	indigestion

PART II: A SURVEY OF THE INCIDENCE OF RECURRENT PAROXYSMAL HEADACHE

Migraine is but a symptom and undoubtedly may be due to a variety of causes, one of which appears to be allergy. This being the case, one would anticipate rather frequent incidence of this symptom in individuals suffering primarily from other allergic manifestations. A series of 85 consecutive allergic patients, complaining of asthma, hay fever, urticaria, eczema, or gastrointestinal allergy, seen during the winter of

1933-1934 were carefully questioned concerning present or past history of recurrent headaches of the migraine type. Twenty-five, or 29.4 per cent, were subject to such headaches. Four, or 16 per cent, of the latter had recognized the foods causing the headaches, from their own experience. The foods mentioned were peanut, apple, watermelon, and onion. Of the 25, 19 had symptoms other than migraine which they had recognized as due to food idiosyncrasy and which they had learned from experience that they could relieve by specific food avoidance. As we shall bring out shortly, the reason that only 4 out of 25 had previously recognized the foods responsible for their headaches is probably the fact that with the remainder, the responsible foods were such as were eaten frequently, often daily, as a consequence of which they would not be suspected.

It would appear that headaches due to food allergy are quite frequent among allergic individuals, occurring in as high as 30 per cent of cases.

I have recently had occasion[15] to make a survey of an entire rural community comprising 508 individuals, in an effort to determine the actual incidence of allergy. Similar previous surveys by Spain and Cooke[16] and by Piness and Miller[17] had shown that upward of from 7 to 10 per cent of the population suffer from frank allergy. In our own survey 10.8 per cent of the entire population were recorded as frankly allergic. This corresponds very well with the two earlier surveys; although we had included as frankly allergic certain groups not included in those, notably recurrent headaches definitely due to specific food sensitization, and clearly recognized cases of contact allergy.

Prior to the survey we had gained the impression that if one were to include minor, transient or evanescent allergic manifestations, not sufficient to require medical consultation, the percentage of allergy in the population would be very much higher. Examples would be those such as the individual who at some time has experienced a brief attack of urticaria, when he suspected some certain food but in which the urticaria did not persist; or an unexpected food disagreement causing nausea or diarrhea, colicky cramps, or even vomiting; or explosions of sneezing as upon arising in the morning, enough to make one wonder whether one is going to get hay fever; or headaches or dizziness in which one wonders concerning a certain food; or dermatitis or other irritation of the skin due to contact with wool, etc.; or sensitiveness to certain medicines, such as quinine, aspirin, or iodides; or particularly the individual who has had one or more of these experiences and has had the opportunity to try out the subject of his suspicions often enough to convince himself definitely of its etiologic importance. Thereafter he has avoided the causative factor and has remained free from symptoms.

We found in the survey that in addition to the 10 per cent frankly allergic, 48.1 per cent had had some minor allergic manifestations at

some time in the past. The interesting fact derived was that almost without exception these persons could name the substance responsible for symptoms. In other words, they fell into the last group just mentioned, and this was taken as a criterion for considering them mildly allergic. I have classified the 10 per cent group and the 48 per cent group, *major and minor* allergic individuals or *frank and mild* allergic persons or *"unfortunate" and "fortunate."* The latter terminology is based upon the observation that a minor allergic individual is fortunate enough to become sensitized to some allergen with which he comes into only occasional contact and therefore has no difficulty in recognizing and avoiding the cause, thereby curing himself without requiring a physician's services. The unfortunate or major allergic has had the misfortune of becoming sensitive to something with which he comes in daily or frequent contact and which he has great difficulty in avoiding. The minor allergic knows the cause and cures himself. The major allergic does not know the cause and must be studied allergically.

Nine and one-half per cent of the community gave no history of symptoms that could be interpreted as allergic manifestations. Eleven and nine-tenths per cent gave questionable histories. They may have had some symptom that might have been interpreted as allergic, but they could not name the cause. It would therefore have been risky to classify them as even minor allergic individuals.

Among the 508 persons in the community, 168, or 33 per cent, suffered from chronic or recurring headache. Thirty-six, or 7 per cent, of the population had found that their headaches were due to the eating of certain foods, and without exception they could name the foods responsible therefor. Thirty-nine, or 7.35 per cent, attributed their headaches to definite causes, listed as follows: constipation, high blood pressure, gall bladder disease, fatigue, indigestion, sinus disease, eyestrain, asthma, catamenia. The remaining 91 were unable to explain their headaches. Seven per cent of the total population had headaches due to food allergy. Twenty-one and four-tenths per cent of the 168 individuals with chronic headaches had found them due to food allergy. Foods listed as responsible are shown in Table IV. It will be seen that with the exception of milk and possibly potatoes the responsible foods are such as are eaten only occasionally.

TABLE IV

FOODS FOUND BY EXPERIENCE TO CAUSE HEADACHE BY 36 PERSONS IN A SURVEY OF A HOMOGENEOUS POPULATION OF 508. FIGURES INDICATE NUMBER OF PERSONS

Onion	13	Cucumber	2
Watermelon	13	Milk	2
Cabbage	9	Spinach	1
Pork	3	Pear	1
Strawberry	2	Peach	1
Peanut	2	Brunswick stew	1
Irish potato	2	Pineapple	1

That the type of headache under consideration is rather more frequent among allergic than among nonallergic individuals is indicated by the fact that of the entire population (508) 58.9 per cent had major or minor allergy. Among the 168 with headaches, 72.6 per cent were classed as major or minor allergic individuals.

In defense of the accuracy of these statistics, one must not infer that all persons with headaches who were classed as allergic were put down as having headache due to food allergy. As stated above, the 7 per cent of the population with allergic headaches include only those who could name the responsible food. There were many persons classed as allergic on account of other manifestations who at the same time suffered from headaches, cause unknown or otherwise explained. These are not included in the list of allergic headaches. Of those with recurring headaches who were unable to ascribe a cause, 14.4 per cent were major allergic persons and 48.3 per cent minor allergic persons. Of those with headaches due in their opinion to other nonspecific causes 7.8 per cent were major allergic individuals, 56.8 per cent minor allergic individuals. These are not included in the series of allergic headaches, although in all probability a certain proportion of them are actually such.

If, as appears, 7 per cent of the entire population suffers from headaches due to food idiosyncrasy and 30 per cent of frankly allergic individuals have headaches from the same cause, the subject is one which merits clinical attention and which justifies attempts to improve the results.

PART III. DISCUSSION OF METHODS FOR IMPROVING THERAPEUTIC RESULTS

In our 1933 survey we found evidence of an allergic factor in at least 68 per cent of our migraine cases. We have just seen that among 168 cases from a homogeneous population with recurrent paroxysmal headache, 72 per cent were allergic. The coincidence of these two independent figures is striking and gives us reason to believe that allergy is a factor in upward of 70 per cent of so-called migraine. While we have succeeded in giving reasonable relief to 50 per cent, we should strive to increase this figure to 70 per cent at least.

We have seen that the character of the migrainous headache offers no prognostic information other than that in the long run unilateral headaches are more likely to respond well. Assuming the absence of an organic pathologic condition, the only outstanding nonallergic factor appears to be a regular periodicity, especially that associated with the catamenia. In the limited number with accurate records of family incidence of migraine, it would appear that the heavier the family incidence, the better the results to be anticipated.

Sex appears to be a factor to a degree, since 48 per cent of females were relieved as against 60 per cent males. The reason is conjectural.

It might be associated with endocrine disturbance or due to the fact that the male, not usually in the kitchen and at card parties, suffers less temptation to err from his diet.

In our 1933 survey we observed that not the age of the patient, nor the age of onset of migraine, nor of the duration of this symptom had any effect on the prognosis.

Nonallergic excitants, such as constipation, emotion, fatigue, probably play the most important part in the perpetuation of symptoms, the degree of importance varying in different cases. I must admit that we have paid little attention to these other factors, since the patient as a rule had previously received ample instruction thereon and was avoiding them as well as the exigencies of his or her daily life permitted. Our primary interest was entirely in the allergic aspects. Further stressing of the importance of these nonspecific factors would probably improve the results to a degree.

The matter of cooperation is a very decided factor. Many of the patients with poor results have been unwilling to stick to their dietary program over a sufficiently long period. The study is not completed with the application of the sensitization tests but requires a long period of collaborative observation between patient and physician, often with the use of trial diets and the keeping of a food diary, through a period covered by the occurrence of a number of successive headaches, before completely satisfactory results are obtained. From the allergic point of view this continued collaboration is one of the most important factors governing final success. Among those whom we followed for less than six months 42 per cent were adequately relieved, whereas of those followed for more than eighteen months 65 per cent obtained such relief.

Skin Tests.—The skin tests, even when scratch and cutaneous methods are combined in the study, failed to give reliable information in 50 per cent of food reactions. However, since it does give reliable information in the remaining 50 per cent, I have found it the best starting point for an allergic study, following a very detailed discussion with the patient of his allergic symptomatology, experiences, and suspicions. Two important points concerning the reliability of the skin tests developed during our 1933 survey. The first was that the borderline skin reactions may be of as much importance as the strongly positive reactions. For this reason one should pay attention to the borderline and delayed reactions. The second was that positive reactions may occur to foods which are not actually causing allergic symptoms. This applies not only to the borderline but also to some of the strongly positive reactions. For every 100 positive or borderline reactions to foods an average of 50 were actually found to cause symptoms. The logical procedure in the study of a case is, therefore, to have the patient avoid all positive and borderline reactors so far as is consistent with general dietary principles. Then, as the patient is relieved, successive foods are added to the diet. In this way the actual causative foods are discovered.

Trial Diets and the Food Diary.—Following the skin test the patient is placed upon an appropriate diet in which the positive-reacting foods are eliminated, together with such foods as he may have discovered in his own experience cause trouble. Since there may be still additional foods, he is instructed in the keeping of a food diary in which the foods eaten at each meal are recorded and checked against the days with headaches. In this way additional foods which had failed to give positive cutaneous reactions are discovered. For every 100 actually causing trouble and discovered by the method of skin testing, we have found 40 foods by the method of food diary. Failure on the part of the patient to continue cooperation and study, after the original testing and inauguration of the preliminary program, may therefore be expected to diminish materially the percentage of good results.

The Leucopenic Index.—While the cutaneous sensitization reaction has been of inestimable value in the study of allergy and has been responsible for the major advances in our knowledge thereof, all who are seriously interested in allergy will agree that it is in no sense infallible. This was exemplified, as has just been brought out, in both the false positive and the false negative reactions. The food diary has been of material assistance, but this again is not infallible. Its analysis sometimes presents unexpected difficulties and the factor of the patient's continued interest and cooperation is one that is sometimes difficult of control. I have felt the need of some additional diagnostic procedure, particularly in the food allergies, which could be used with and correlated with these other methods.

Some years ago Widal, Abrami, and Jancovesco[18] proposed a test for liver function which they designated the hemoclastic crisis or colloidoclastic crisis test. The test was based upon the presumption that the liver normally prevents the entry of absorbed foreign protein from the portal circulation into the general circulation. The test consisted of the feeding of 200 c.c. of milk, before and after which white counts, clotting time, and blood pressure were recorded serially. I, along with many others, found after serious trial that this was not a reliable test of liver function. I did, however, observe that not infrequently allergic individuals, even with normal livers, reacted positively, with leucopenic reaction, drop of blood pressure, and delayed clotting time following the ingestion of milk in the postabsorptive state. The obvious explanation, in view of the fact that the anaphylactic reaction is usually accompanied by leucopenia, was that such allergic persons were sensitive to milk.

Recently it occurred to me that if this were true, such a reaction might be characteristic of sensitization to various foods, in a specific way. If this were true, it would make no difference whether a postprandial leucocytosis actually exists or not, as is claimed by Garrey and Butler,[19] provided normally there is no postprandial leucopenia.

During the last nine months I have been making a study of the incidence of digestion leucopenia and changes in the blood pressure and clotting time following the postabsorptive ingestion of specifically allergenic foods. Up to the present 250 such tests have been made. While this will be a subject for separate reports, it is of interest to state here that the curve of leucocyte response following the ingestion of allergenic foods often shows a drop, which is not the case following the ingestion of foods to which the individual is not sensitive; that this has been found often to be of diagnostic importance, where the skin test has failed to show evidence of sensitization; and that, in the study of the patient with allergic migraine, it is especially likely to be of assistance in cases of sensitization to staple foods such as wheat, egg or milk—cases in which the skin test and the food diary are especially likely to fail to give the desired information. It would appear to be a diagnostic method which, used in conjunction with the skin tests, gives additional information on food allergy without the necessity of depending upon the equation of the patient's cooperation.

CONCLUSION

1. True migraine is often due to food allergy. Upward of 70 per cent of recurrent paroxysmal headache without organic pathologic condition is associated with allergy.

2. At least 7 per cent of a homogenous community was found to have suffered at one time or another from recurrent headaches due to specific food idiosynerasy.

3. Such headaches appear to be more frequent in allergic individuals than in the population at large.

4. Factors which appear to influence results obtained in the treatment of allergic migraine are: the period of supervision; the cooperation of the patient; occurrence of headaches with the catamenia; and a proper utilization of the available diagnostic measures, notably the skin test, the food diary, and the leucopenic index.

The evidence would indicate that with the allergic diagnostic and therapeutic methods now available, properly employed, treatment should be successful in at least 70 per cent of cases of migraine.

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THE SIGNIFICANCE OF TOBACCO REACTIONS IN ALLERGIC CHILDREN*

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IN THE last year or two a great deal of attention has been called to the incidence of tobacco reactions, especially in cases of thromboangiitis obliterans. Harkavy and his associates[1] reported 68 cases of thromboangiitis obliterans varying between the ages of twenty-four and fifty-six years and 122 controls. Out of 68 cases of thromboangiitis 58, or 83 per cent, reacted to tobacco extract. Ten, or 17 per cent, were negative. Of 107 controls, all smokers, 97 were negative and 10, or 10 per cent, reacted to tobacco. In none of the 10 per cent were the reactions marked.

This observer further reported definite skin reactions to foods and to inhalants in 15 of the thromboangiitis obliterans cases. He further reported that of 20 serums taken from patients sensitive to tobacco, 13 gave positive passive transfers. The conclusion is drawn that these individuals were definitely atopic and that the phenomena were true antigen-antibody reactions.

Sulzberger[2] reported that the tobacco extracts contained little nicotine, and he reported his findings on eighteen patients; nine of whom suffered from thromboangiitis obliterans, one from rheumatic heart disease, one from arteriosclerosis, one from precordial pain, one from questionable tobacco neuritis, three from hypertension and migraine, two from migraine, and three controls. Of his nine thromboangiitis obliterans cases, he obtained a three-plus reaction in only one and in four only a two-plus reaction and in three only a one-plus reaction. The rest of the cases gave either one- or two-plus reactions. With nicotine he failed to obtain more than a one-plus reaction and that in only four patients. Passive transfer was successful to tobacco in one case and unsuccessful in another.

Sulzberger[3] reported another series of 24 cases with typical thromboangiitis obliterans, 19, or 78 per cent, gave positive wheals from one-plus to three-plus. He reported 36 per cent positives in a control group. He concluded that tobacco smoking brings about a sensitization of the superficial layers of the cutis to tobacco extracts and that a markedly higher percentage of the persons affected with thromboangiitis have a sensitized vascular system than is to be found in other

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smokers. In one case he was able to transfer the tobacco sensitivity. He concluded that the sensitization in these cases is nonatopic.

Harkavy[4] reported that 30 per cent of his controls reacted to tobacco pollen and seed while 38 per cent reacted to the leaf. In the thromboangiitis group 59 per cent reacted to the pollen and seed in contrast to 78 per cent to the tobacco leaf. He reported no instance in his controls of any exclusive reactions to tobacco pollen or seed without a simultaneous reaction to other allergens, such as tobacco leaf, ragweed, timothy, or horse dander.

Harkavy and Romanoff[5] concluded that tobacco extracts prepared from cured leaves are comparable in their allergenic constancy with those of ragweed, timothy, and horse dander. They concluded further that the 69 per cent positive skin reactions in thromboangiitis obliterans cases as compared with 11 per cent in the controls would seem to indicate that of the substances tested, tobacco is the dominating reacting allergen in thromboangiitis obliterans.

For this study we took 53 unselected cases in our children's asthma clinic at the New York Post-Graduate Hospital. All these children were allergic in some way, either to proteins, that is to food or inhalant substances, or to the products of bacteria. These were children who had never smoked or had contact with tobacco except in the most casual way. We felt that if these children showed sensitivity to tobacco to any degree, the interpretation would be open to question. The ages of the children varied from three to twelve years. There were 43 male children and 10 females. They were tested intradermally with a tobacco extract containing 0.1 mg. of nitrogen per cubic centimeter. The extract was made from Virginia leaf tobacco which had been defatted and extracted in the usual way, and dialyzed to remove as much of the coloring matter as was possible. Care was taken not to use more than 0.01 c.c. in performing the test. Of the 53 children so tested only six gave negative reactions. Two of these children were twelve years of age. There were 19 slight reactions, 22 moderate reactions, and 6 marked reactions. Of those who gave marked reactions, one was twelve, one was eleven, one was ten, one was nine, one was five, and the other was four years of age. The six patients negative to tobacco on skin tests were analyzed, as is shown in Table I, for their skin sensitivities if any. Three were definitely allergic to other substances, and three were cases of bacterial allergy. Table II shows the result of analyzing the six patients with marked skin reactions. One was a case of urticaria and gave moderate skin reactions, the significance of which could not be clinically demonstrated as a cause of the patient's urticaria. The interpretation put on these food reactions, which were only moderate in type, was that they were

purely nonspecific and of no significance. The remainder of the patients gave multiple skin reactions. Three were pollen cases and two were sensitive to dust and the cereals.

TABLE I

PATIENTS NEGATIVE TO TOBACCO

PATIENT	AGE IN YEARS	SEX	OTHER SKIN REACTIONS
J. P.	12	Male	Dust (moderate)
M. E.	7	Female	Dust, ragweed (moderate skin reactions)
S. G.	9	Male	Dust (moderate), horse serum (positive)
B. R.	12	Female	Infective asthma, negative skin tests
I. M.	3	Male	Infective asthma, negative skin tests
G. S.	6	Female	Infective asthma, negative skin tests

TABLE II

MARKEDLY POSITIVE TOBACCO REACTIONS

PATIENT	AGE IN YEARS	SEX	OTHER SKIN REACTIONS
I. W.*	12	Male	Trees, timothy, ragweed, dust
G. S.	5	Male	Timothy, ragweed, dust, horse epithelium, horse serum
H. A.	4	Male	Urticaria with moderate skin reactions to milk, eggs, barley, rice, lima beans, green peas
L. Q.*	10	Female	Dust, barley, oats
E. M.*	11	Male	Dust, rice, tomato, cocoanut
M. M.*	9	Male	Ragweed, dust

*Patient giving marked plus reaction, serum used for passive transfer.

The four most marked were selected for passive transfers according to the Prausnitz-Küstner method. The recipients for the transfers were extremely difficult to find due to the frequency of the moderate reactions. Each serum was tested on three different individuals negative to tobacco. Two of the children with positive reactions to tobacco were sensitive to ragweed. In both cases the reaction to tobacco could not be transferred, while the reaction to the ragweed was transferable. The two other children were not pollen sensitive and both gave reactions to dust and the cereals, and in these two children likewise the tobacco reaction did not transfer.

When we compare these findings with Harkavy's control findings of 11 per cent, we find a marked variation. We find first approximately 12 per cent giving very marked reactions intradermally and 41 per cent giving moderate reactions of about two- or three-plus and 35 per cent giving slight or one-plus reactions. In other words, only 11 per cent of the entire group were flatly negative. The striking disparity between these figures and those of the aforementioned observers must make us wonder whether or not the reaction has any great significance or value.

In addition various strengths of tobacco extract were tested on guinea pig uterine strip using the Dale technic. No positive findings were found; so we may conclude that tobacco extract is not irritating to the uterine muscles of a guinea pig, and therefore does not contain any histamine-like substance.

In conclusion, then, we may state that we saw many reactions in allergic children who have never smoked and one in a child of three years which was very marked in character. The significance of these reactions is open to question, but in view of the absence of transfers and in view of the frequency of the reaction in children, great caution must be used in interpreting its value as a diagnostic test of any importance. The importance of any positive skin reaction in any allergic condition can only be interpreted as having significance when the clinical test proves its importance. Obviously, in children ranging from three to twelve years, the clinical importance of tobacco leaf is negligible. Therefore, the occurrence of this reaction must be interpreted in the light of many other reactions, such as morphine and histamine, the importance of which cannot be checked clinically.

CONCLUSIONS

1. Of 53 unselected asthmatic children tested to tobacco extract, only 6 were negative, i.e., only 11 per cent.

2. There were 19 slight, 22 moderate, and 6 marked skin reactions to tobacco.

3. Of the children negative to tobacco extract, three gave other skin reactions, including one to ragweed, and three were cases of bacterial allergy.

4. Of those children reacting markedly to tobacco, one was a case of urticaria with food reactions, and the rest gave multiple skin reactions.

5. Testing the tobacco extract on uterine strip of guinea pig using the Dale technic was negative.

6. This reaction was elicited in nonsmokers, children from the ages of three to twelve years. What its significane is, is not known, and any rigid interpretation of the skin reaction may lead us into error. It is probably analogous to what we see when we test patients with solutions of morphine and histamine.

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30 WEST FIFTY-NINTH STREET

HYPERSENSITIVENESS TO INSULIN

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VARIOUS types of insulin sensitiveness have been described.[3, 4, 7] Most of these reactions have occurred in diabetic individuals without personal or family histories of allergy.

The work of Dr. J. A. Murphy and his associates[6] on insulin sensitivity with attempted passive transfer is of particular interest in relation to the present experiment. Both of their cases were in diabetic patients; one developed urticaria, the other asthma, following a series of insulin injections.

J. R. Williams[1] described two cases of insulin allergy in diabetic persons. One patient had edema of the eye and gastrointestinal symptoms which were relieved by changing from pork insulin to beef insulin. The other patient, on being tested, gave a marked reaction to pork insulin lasting twenty-four hours. The beef insulin reaction lasted twenty minutes. The patient was relieved by using beef insulin.

L. M. Bryce[2] found a diabetic patient who was sensitive to all types of insulin and succeeded in producing a degree of tolerance by giving the patient graduated doses of insulin. She demonstrated the sensitiveness by both the direct and the indirect methods of testing.

In the case we are about to describe, of a nondiabetic individual, apparently the patient has a reagin mechanism for insulin, both for the animal and for synthetic forms.

CASE REPORT

On May 7, 1934, Mrs. L. L. was admitted to the medical service of the Fifth Avenue Hospital, and was under the care of Drs. C. F. Tenney and T. T. Mackie. She was under treatment for ulcerative colitis. This diagnosis was confirmed by x-ray examination, which showed typical findings.

The significant laboratory findings were as follows: The hemoglobin ranged from 59 per cent to 68 per cent. Red blood cells ranged from 3,800,000 to 4,400,000. White blood cells were 11,000, with eosinophiles 10 per cent. The blood sugar was 112 mg. per cent.

Because the patient was so much underweight, it was decided to give her small doses of insulin. On May 16 she received 10 units of insulin (Lilly) and immediately developed severe urticaria beginning around the site of the injection and spreading over the entire body. This attack lasted four hours. The next day five units were given

*From the Department of Medicine, Asthma Clinic, Fifth Avenue Hospital.

which did not produce any reaction. On May 23 the patient again had 15 units of insulin (Lilly) and a few hours later developed severe urticaria. These attacks were relieved by injections of adrenalin.

The patient gave the following history of allergy. She had had eczema in childhood, and has had hay fever for many years during the ragweed season. She has never had any treatment for the allergic condition. A cousin on the maternal side had severe eczema; her brother had eczema, and her father had hay fever.

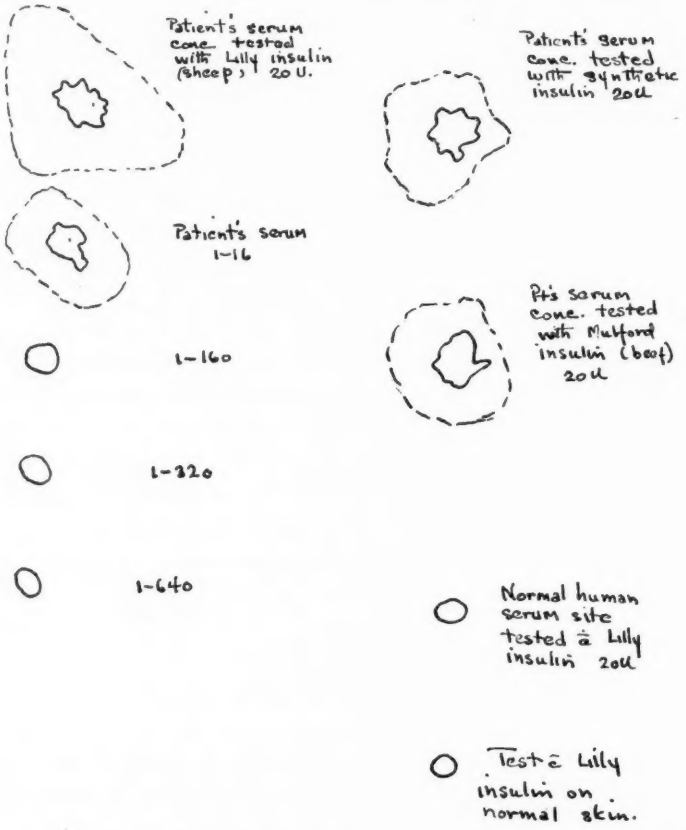
Patient's serum conc. tested with Lilly insulin (sheep) 20 U.
Patient's serum conc. tested with synthetic insulin 20 U
Patient's serum 1-16
1-160
Pt's serum conc. tested with Mulford insulin (beef) 20 U
1-320
1-640
Normal human serum site tested à Lilly insulin 20 U
Test à Lilly insulin on normal skin.

Fig. 1.—Passive transfer of insulin hypersensitiveness. Left-hand row represents reactions at sites injected with dilutions of patient's serum and reinjected with 0.02 c.c. of insulin.

It was decided to do a complete set of intradermal tests including those for various types of insulin. She was tested intradermally with Lilly insulin (sheep), Mulford insulin (beef), and synthetic insulin which was very kindly given to us by Dr. W. C. Spain. In a few minutes each one of these tests showed a marked reaction (wheal ½ inch in diameter with pseudopods). Tests with inhalants showed positive reactions to ragweed, dust, and goat epithelium. All the food tests were negative.

Thirty c.c. of blood was taken from the patient on August 5, and the serum was sterilized by passing through a Seitz filter. A young male, twenty-four years old, non-sensitive, was used as recipient for all transfer tests. The results of the direct intradermal tests were checked by means of the Prausnitz-Küstner transfer on this recipient.

TITRATION OF REAGINS

The patient's serum was diluted 1:16, 1:160, 1:320, and 1:640 with physiologic saline. An injection of 0.1 c.c. with each dilution was

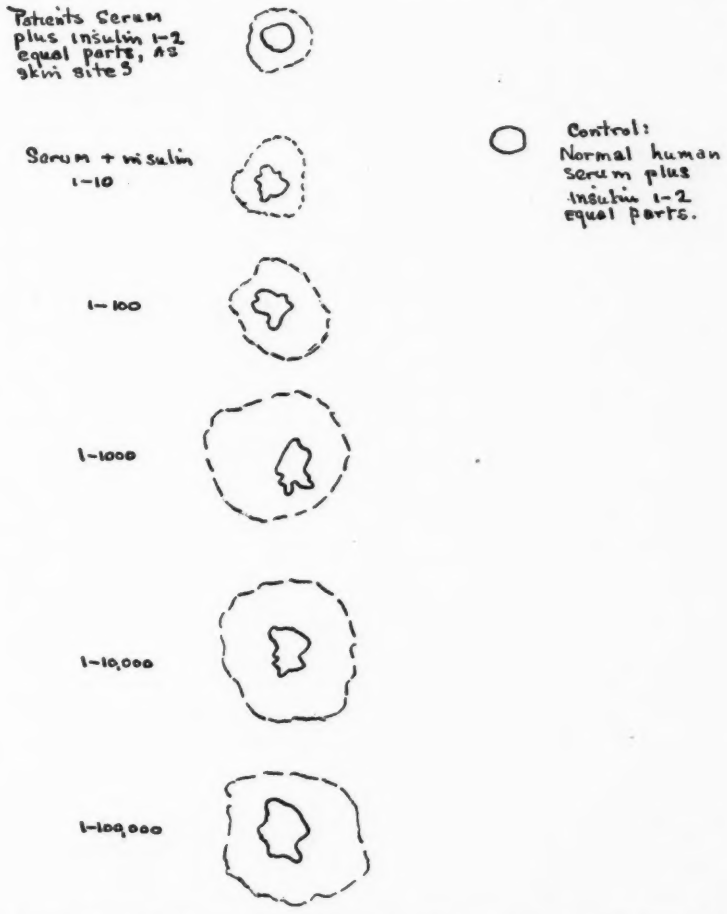
Patients Serum plus insulin 1-2 equal parts, as skin site 5
Control: Normal human serum plus insulin 1-2 equal parts.
Serum + insulin 1-10
1-100
1-1000
1-10,000
1-100,000

Fig. 2.—Passive transfer of insulin hypersensitiveness. Sites injected with 0.1 c.c. of patient's serum and retested with 0.02 c.c. of insulin.

made on the right arm of the recipient. Three more injections were made with concentrated serum of the patient and one more injection with normal human serum, on August 7, 1934. On August 9, all but two of these sites were tested with Lilly (sheep) insulin 0.02 c.c. The remaining two sites were tested respectively with synthetic insulin

0.02 c.c. and beef insulin 0.02 c.c. All types of insulin were 20 U to the cubic centimeter. (Fig. 1.)

The synthetic, Lilly and Mulford (beef) insulins showed about equally marked reactions when tested at the sites of the concentrated patient's serum. At the site of the normal human serum no reaction occurred, and the control in recipient's skin was negative. The patient's serum diluted 1:16 gave a marked reaction when the site was tested with 0.02 c.c. insulin (Lilly). The sites sensitized with higher

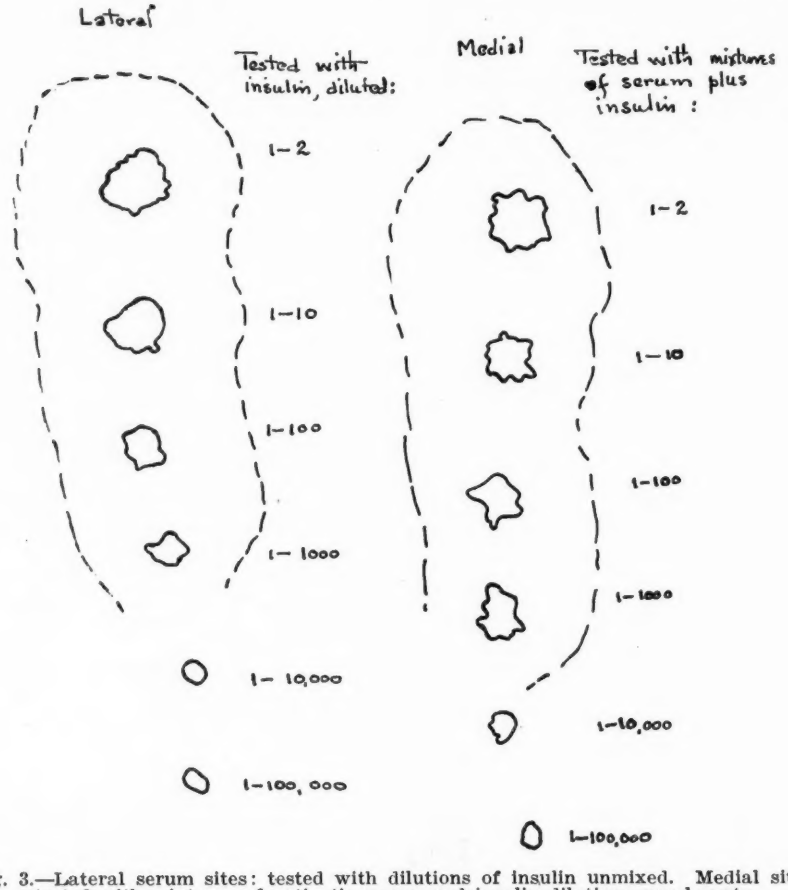

Fig. 3.—Lateral serum sites: tested with dilutions of insulin unmixed. Medial sites: tested with mixtures of patient's serum and insulin dilutions equal parts.

dilutions of the patient's serum did not show any reaction when tested with Lilly insulin.

NEUTRALIZATION OF REAGINS

The patient's serum was mixed in equal parts with dilutions of insulin as follows: 1:2, 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000. One-tenth c.c. of each mixture was injected at sites on the left arm of the recipient. These in turn were tested with 0.02 c.c. of insulin (Lilly) after forty-

eight hours. As a control, normal human serum mixed with insulin 1:2, was employed.

The result showed a typical neutralization of reagins, the patient's serum which was mixed with insulin 1:2 showing a very small reaction, and the serum mixed with higher concentrations showing marked reactions. (Fig. 2.)

The controls used in this part of the experiment were dilutions of insulin with saline, as above, unmixed with serum. Therefore, they were 50 per cent stronger than the insulin-serum mixtures.

Injections were made in two rows of six each with the patient's serum, 0.1 c.c. in each site. In forty-eight hours one row was tested with the insulin and serum mixtures and one with the insulin dilutions. There appeared to be no distinct difference in the reactions obtained with the serum and insulin mixtures as compared with the insulin dilutions, showing that so far as we can tell by this method of testing, when the neutralization takes place the antigen (insulin) seems to be unchanged. (Fig. 3.) We expected that the unmixed insulin dilutions would show larger reactions, being 50 per cent stronger.[5]

Each time, before the recipient was tested with insulin, he was given a large glass of orange juice with sugar to prevent any untoward reaction from insulin absorption. We were not able to repeat these experiments with other forms of insulin as we would have liked, because of difficulty in obtaining more serum. It also would have been interesting in this case to do a precipitation titer. However, the insulin and serum mixtures which were allowed to stand at room temperature for twelve hours before being used did not show evidence of precipitation.

SUMMARY

A patient developing severe urticaria on two occasions, after a small dose of insulin, was tested and was found to have reagins in her blood for the animal and synthetic forms of insulin, and for other substances as well. Three types of insulin gave positive reactions, both by direct and by indirect tests in this patient. Her serum showed a good concentration of reagins for insulin as demonstrated by the titration test. These reagins were neutralized by the corresponding antigen, which appeared to be unchanged after neutralization took place.

In view of the findings in these experiments it is suggested that a patient giving a personal or family history of allergy be tested for insulin before treatment is begun with it.

As a control, 11 hay fever patients at the Fifth Avenue Hospital Clinic were tested by direct test with the three forms of insulin. One of these gave moderate reactions to two kinds. The other patients gave negative or slight reactions.

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COTTONSEED ALLERGY AND GIN*

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GIN has been mentioned among the numerous commodities which may contain allergens of the cottonseed. Brown,[1] in describing the manufacture and usual uses of cottonseed oil, states that this oil is also employed occasionally in the preparation of gin. Bowman and Walzer[2] apparently give some credence to information that the seed of the cotton plant is sometimes used in the manufacture of gin. Vaughan,[3] by citation of a personal communication, reports one instance of an allergic reaction attributed to the drinking of "cottonseed gin," and crediting the same authority mentions[4] cottonseed among other more familiar raw materials entering into the manufacture of fermented and distilled beverages. Feinberg,[5] in a text of recent issue, asserts that cottonseed is sometimes used in the manufacture of gin.

Inquiry developed the fact that allusions to the possible or probable occurrence of cottonseed allergens in gin were in some measure founded upon a reported hypersensitiveness to gin among a few individuals who were also declared hypersensitive to cottonseed on the basis of test and history or history alone. Since these reactions were attributed to some cottonseed component of the beverage, an attempt was made to trace the reports that cottonseed or some product of the cottonseed is sometimes used in the manufacture of gin. The results of this inquiry led only to rumor or, in one instance, to an impasse when A referred to B as the informant and B, in turn, credited A as the source of information about use of cottonseed in gin manufacture.

Gin is widely regarded as an alcoholic beverage of relatively high proof carrying the flavoring principles of juniper berries. This conception can be reconciled with the early history of the origin and development of gin manufacture in Holland, but the inclusion of other extractives in gin either as a substitute for juniper or more frequently as a desirable supplement to this flavor has long been an accepted trade practice. Whether some product of the cottonseed is or has been used in the preparation of gin was of sufficient consequence to carry this investigation to a satisfactory conclusion. The results seem worthy of record for the information of allergists.

A search of the literature provided a variety of formulas and descriptions relating to the technology of gin manufacture, but no refer-

*Protein and Nutrition Division, Bureau of Chemistry and Soils, United States Department of Agriculture.

ence to cottonseed or a product of the cottonseed was found among these sources. Consultation with Dr. James M. Doran† and officials of the Federal Alcohol Tax Unit,‡ and inquiry among manufacturers of gin and gin flavors and among dealers in the botanicals employed in the manufacture of gin, brought no information about the use or utility of any product of cottonseed in any phase of the manufacture of gin. This investigation did, however, reveal the fact that gin is usually of complex and variable composition containing the rectified spirit from barley, malt, rye, wheat or corn and the flavoring principles of some combination of aromatic plant materials. From various sources the following comprehensive but, presumably, incomplete list of usual and unusual sources of the flavoring ingredients of gin has been assembled. The items preceded by an asterisk have been reported as being allergenic or have appeared in some list of allergens:

*Almond meal
Angelica root
Angelica seed
*Aniseed
Buchu leaves
Calamus root
*Caraway seed
*Cardamom seed
*Cassia (cinnamon)
*Cayenne
*Clove
*Coriander seed
Fennel seed
*Ginger root
Grains of paradise (unofficial variety of cardamom)
*Hop flowers
*Juniper berries
*Lemon (derivatives of fruit)
*Licorice root
*Mace
*Nutmeg
*Orange, sweet (derivatives of fruit)
*Orange, bitter (derivatives of fruit, blossoms and other tissues)
*Neroli (from blossoms)
*Petitgrain (from leaves and twigs)
*Orris root
Rosemary
Sloe berries
*Turpentine
Wormwood (prohibited in beverages imported or manufactured for sale in the United States)

Some gin may be flavored only with the derivatives of juniper berries, but most formulas and current practices direct the combination of several (from three to ten) of the listed flavoring components for one brand or variety of gin. The botanicals or source materials for the flavoring may be extracted concurrently with the fermentation or distillation stages of the manufacture, or the prepared extractives may be added to the rectified spirit. One favored method is to combine the botanicals into a "gin mat," supported in the head of the gin still, from which the distilling vapors collect and carry the extractives into the condensate. When legal restrictions limited the

†Secretary, Distilled Spirits Institute, Washington, D. C., formerly Commissioner, Bureau of Industrial Alcohol.

‡Alcohol Tax Unit, Bureau of Internal Revenues, Washington, D. C.

use of ethyl alcohol, the readily available "gin drops" or flavors were, in some instances, reported to be sold in a vehicle of isopropyl alcohol.

One fact, perhaps apparent but worthy of declaration, is that no product of the cottonseed appears to be known as a usual or unusual ingredient in, or adjunctive to, the manufacture of gin, as either an industrial or a private enterprise. This view has been repeatedly submitted both by those consultants conversant with some phases of gin manufacture and by authorities on the production, milling, refining, and utilization of cottonseed and its derivatives. Scarcely conceivable, indeed, is the use of this seed, which is high in both protein and oil, and capable of providing only a low and relatively very costly proportion of fermentable carbohydrate for the purpose of alcohol production; an organoleptic evaluation of a fermenting mash containing cottonseed, the press cake or the oil-free residues of cottonseed will corroborate theory in this instance. The oil from the cottonseed is devoid of aromatic flavoring principles and when refined for edible uses is yellow in color, possesses little flavor, and is virtually insoluble in a hydroalcoholic solution such as gin. If cottonseed and cottonseed oil have virtues to commend their use in the manufacture of this beverage, they have not received recognition.

Most rumors may possibly be credited with truthful origins. In this investigation, one properly accredited item of evidence came to light to suggest that reports of cottonseed in gin may have arisen from an honest confusion of terms. A southern manufacturer while advertising in cotton trade papers the virtues of the D—e Gin was rendered notice by a northern distiller of alcoholic beverages that the name D—e was already registered and employed in the brand name for gin. The conflict between the two claimants for the same trade name arrived at an amicable settlement upon mutual recognition that the one D—e Gin identified a machine for separating cottonseeds from cotton lint, and the other identified a gin which was an alcoholic beverage.

SUMMARY

A satisfactory basis for attributing allergenic properties of the beverage, gin, to allergens of cottonseed is not found in an examination of the available information concerning the technology of the manufacture of gin nor in the consideration of the properties and known uses of cottonseed, the edible oil or other products of cottonseed.

A brief description of the technology of the manufacture of gin and an assembled list of the components or adjuncts sometimes employed in the manufacture of this beverage are presented.

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ASTHMA PRODUCED BY SUSCEPTIBILITY TO UNUSUAL ALLERGENS

Linseed, Insects, Tobacco, and Chicory*

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WE HEREBY refer to some cases observed by us in which asthma was due to susceptibility of the organism to unusual allergens. Although similar sensitivities have been described by other authors, these remarks further show that such possibilities should be taken into consideration.

SUSCEPTIBILITY TO LINSEED

The three cases observed by us were found in chemists and in druggists. The frequent use that we make in this country of poultices in the treatment of severe colds, and in the application of damp warmth, and the fact that the poultices invariably are linseed, explain the importance of this allergen to those chemists who are obliged to deal in it. Of the three following cases, only one gave suspicion that clinically linseed might turn out to be the origin of his asthma.

Case 1.—R. M., subject to severe colds since infancy. While suffering one of these, a poultice was applied which caused him severe burns. From that time he suffered severe asthmatic paroxysms whenever he took a cold, especially on applying the linseed poultices. Suspecting these to be the cause, we omitted them, and the patient improved. After some years he became a chemist and established a shop in one of the provinces. He then began to suffer constant attacks which were very intense. These disappeared on his return to Madrid. No pulmonary or circulatory lesion was found on examination. All dermal reactions were negative except that of linseed, which gave a strong positive reaction, and the clinical manifestations of his asthma became accentuated after the test. Some positive bacterial reactions were obtained intradermally. Passive transfer was carried out (Prausnitz-Küstner), which resulted in a strongly positive reaction to linseed. The patient was instructed as to the cause of his asthma, and a well-isolated place was built close by in which he kept the linseed packed, none of it remaining in the shop. The attacks disappeared. One day on entering his shop he was overcome by a paroxysm of coryza, and it was discovered that some linseed had been placed there as an experiment. Desensitization treatment was successfully carried out.

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CASE 2.—J. R., patient had been in good health until he established a drug store. Soon after, he began to suffer attacks of asthma. These were intense and persistent. He was advised a "change of air." He recovered from the first day in a small village close to Madrid. After a short time he returned to work and the attacks reappeared. He underwent hydropathic treatment and recovered. On again renewing work, he suffered further attacks. Connection with his store was suspected. Experiments with house and store dust were negative. We investigated all organic products (including ipecac) in the department which might produce dust in the atmosphere. Tests were all negative excepting linseed, with which we obtained a very strong positive reaction with provocation of an attack. Passive transfer (Prausnitz-Küstner) was positive.

Provocation Trial: The patient was not allowed to return to the store, and recovered. One morning while he was still asleep, the room next to his was sprinkled with linseed; this caused the patient to awake with a terrible attack of asthma. Desensitization treatment was performed, and even with large dilutions (1:100,000,000) every injection was followed by a paroxysm. One of these caused an eruptive urticaria generalized by extraordinary itching and intense ocular congestion. All this disappeared on further injections of the extract as well as from 0.5 to 1.0 mg. of adrenalin, which increased his tolerance and permitted us to increase the antigenic concentration.

We were successful after treatment in getting the patient to remain in the store without showing asthma. The hypersensitiveness of the patient was revealed by the following curious peculiarities. While undergoing treatment, one morning on entering his house he suffered a severe fit of sneezing followed immediately by an attack of asthma. The cause, on then being inquired into, was found to be that the porter's wife had a sick child and linseed had been bought in order to make a poultice.

CASE 3.—Miss M. A., a young lady chemist from a neighboring village. She was in perfect health until the beginning of her asthmatic trouble. She had very strong paroxysms for a time which obliged her to go away to another village, where she recovered. Intense attacks were subsequently repeated there. Having finished her study, she established herself, but from the beginning suffered strong attacks of asthma. In the investigation all tests were negative but an intensely positive reaction to linseed and to mustard. Passive transfer was positive. On interrogating her anew, we discovered that in all the places she had suffered these attacks it was due to her having lived in the shop, while the times she was free from the paroxysms she happened to live in private houses away from contact with linseed. We advised desensitization treatment, the results of which are not yet known.

These three cases are absolutely probatory ones, in their history and in their skin reactions, from the passive transmission obtained in the three and by the provocative trial. The curative effect from getting away from the allergen, and in two of them by specific desensitization, were typical.

The most predisposed profession to linseed sensitization is that of a chemist, this being the only one in Spain which establishes a reiterated contact with the substance. This susceptibility is acquired by the respiratory apparatus, although in one of the cases it is evident that it was brought about by the skin having been burnt. In other countries where there exists contact with linseed in other professions this susceptibility likewise appears. In North America it is evidently much used as daily animal feed.[1] In the cases published by Nicholson[2] nearly all are peasants except one, who is a nurse. Likewise in those of Brown,[3] the susceptibility was produced through having lived in a village and employing linseed as food for chickens.

The fact that some nutritious products for man are made with linseed as basis makes susceptibility *per os* possible. Black[4] described two cases of serious allergic manifestations by ingestion of "Roman meal." One should always consider the possibility of susceptibility by contact, with various toilet products containing linseed derivatives. Ramirez and Eller[5] found a patient susceptible to hair tonic made from the seed of flax. Rowe[6] described linseed dermatitis in a patient who got well on leaving his farm, where linseed meal was used. Grant[7] gave numerous similar cases found in workmen employed in the linseed oil industry. In these the symptomatology was not asthmatic but cutaneous. Thomas[8] and others mentioned a possible susceptibility to linseed oil as cause of asthma. Thus far we have not yet come across this in painters. A further possibility lies in the ingestion of seeds of flax or their kinds when used as laxatives, some of which are nothing more than seeds covered over with chocolate. Nevertheless, up to the present we have never yet met such a case.

Other authors have mentioned allergic symptoms brought on by linseed. Frugoni and Ancona[9] referred to susceptibility by poultices, as did Duke[10] and Thomas.[8] In the cases of Figley and Elrod,[11] among asthmatic patients susceptible to substances emitted from a mill, 30 gave positive reactions to castor bean, and of these 5 simultaneously to flaxseed. Rackemann[12] mentioned a case of asthma of this etiology in a druggist's assistant. Feinberg[13] also remarked on the possible susceptibility to flax cloths as well as to linseed in poultices.

Briefly speaking, susceptibility to products from the *Linum ussitatissimus* may be brought about by contact (druggists, painters, peasants, and places where it is used as fodder, by toilet products, etc.), through ingestion (food or medicines made from it), or by inhalation (chemists, druggists, peasants, and mill industries). It may determine contact

dermatitis, digestive allergies, urticaria, angioneurotic edema, and asthma. Asthma will be determined in those patients receiving the allergen by inhalation, but seldom, though likewise possible, by ingestion, as we have already said. The most exposed profession in Spain is that of a chemist, as the linseed here is not used for animal feed. In all cases of asthma found in chemists and in druggists one should bear in mind always the possibility of this susceptibility.

SUSCEPTIBILITY TO "CIMEX LECTULARIUS"

Sternberg[14] published a case of asthma in a man thirty-seven years of age in whom there existed as the only cause a sensitivity to bedbugs. His asthma lasted from July to September. The suppression of paroxysms on changing his bedding, and the passive transfer obtained to the specific allergen, verified the sensitivity. We report the following case:

CASE 4.—E. R., twenty-eight years old, well built and in good health formerly, suffered typical asthmatic attacks for some ten years. These made their appearance with the beginning of the warm season and lasted all summer until the end of September. The periodicity of her trouble suggested to us a pollen syndrome. We investigated this with all the extracts, but every one of these gave negative reactions. The tests on the vegetation surrounding the house were equally unproductive.

The patient lived in an old house which was presumably full of parasites, although she denied this. We nevertheless suspected some kind of domestic parasite apart from flies and mosquitoes, to which she gave negative reactions. We experimented with extract of *Cimex lectularius*. To this she gave a very markedly positive reaction, with general symptoms some hours following (sibilant rhonchi and difficulty in breathing). Passive transfer was attained (Prausnitz-Küstner), and the extract was tested on normal individuals with invariably negative reactions. The patient was then instructed about the cleaning up of her lodging. When this was done, we began the desensitization with extract from the parasite already mentioned, each injection being followed at first by an asthmatic crisis which disappeared on mixing antigen and adrenalin. Eventually, complete tolerance and effective results were obtained. This year the patient did not suffer any attacks during the whole summer.

In nonpollen cases one should always bear in mind susceptibility to some special thing related to the season, be it food (fruits of the season, Frugoni[9]; one of us [J. D.][15]), or elements residing in country houses. Among those things which may be responsible is susceptibility to bedbugs, as in the present case, and probably to other insects as well (flies, Parlato,[16] Figley[17]). We have at present another case of

summer asthma not sensitive to either pollens or food, nor referable to any special house dust. The reactions to flies and to bedbugs have been negative. We are at present experimenting with other insects.

SUSCEPTIBILITY TO TOBACCO

Walker[18] mentioned susceptibility to tobacco leaf in factory or store workers. Susceptibility to tobacco smoke seems, on the other hand, to be more doubtful. Several authors, Brown,[19] and Thomas,[8] have mentioned the frequent possibility of susceptibility to tobacco and therefore recommend its inclusion in skin testing. In the case to which we are about to refer, susceptibility to tobacco was the sole cause of asthma.

CASE 5.—P. L., forty-one years of age, had repeated colds for some years back. Lately, the patient began to suffer frequent attacks of asthma which were almost of daily occurrence. He did not know the cause. Skin tests were negative except in some bronchial bacteria which were not convincing. We therefore made further careful inquiries and were able to find the interesting fact that his attacks began to appear shortly after having undertaken the charge of a tobacconist store. This prompted us to experiment with tobacco extract, which gave a strongly positive skin reaction, and we obtained also passive transfer. He was instructed to keep away from the store for a while, and this brought about the disappearance of attacks. As a first therapeutic measure we proposed that he abandon the store. We then lost sight of the patient, and have heard nothing more as regards his further course.

Another similar case is the following, although in this one susceptibility to tobacco dust is added to hypersensitiveness to other allergens.

CASE 6.—M. J., forty years old, owned a tobacco store in a village close to Ciudad Real. She had suffered from colds since infancy, with paroxysms of sneezing especially during wet weather. Lately the colds settled on the chest, followed by fits of coughing, dyspnea and asthma. All this was more intense in her own village, the patient finding herself free from the trouble on leaving the place. The same occurred on her arrival here for consultation. At that time, physical examination of the respiratory tract was negative. Positive skin reactions to *Penicyllium* and wool from her pillows, and strongly positive reactions to the dust from the store and to tobacco were obtained. Dust from her lodging gave a negative test. Interrogating the patient anew, we discovered that the dust from the store on being swept up always produces her attacks.

Another interesting case is the following, in which susceptibility to tobacco dust was brought about in a professional atmosphere, the allergen reaching the highest concentration.

CASE 7.—R. M., twenty-seven years of age, employed in a tobacco factory, had been inclined to colds since the age of four years, especially in the winter. She found that ever since she started work a year ago in the Madrid tobacco factory, she suffered intense irritation of the throat when tobacco was moved about. She also had paroxysms of coughing and intense feeling of fatigue with no relation whatever to bodily activity. Investigation showed strongly positive skin reactions to tobacco extract (everything else was negative). We recommended to her that she abandon her trade, but know nothing more about her case.

SUSCEPTIBILITY TO CHICORY

This constitutes another possibility, although not a usual one. Chicory (*Cichorium intybus*) is a composition employed in certain regions as food for animals, in litters, and in fertilizers. In Spain its chief consumption is in the form of toasted roots added to coffee in order to render to its infusion certain stronger qualities of taste and color. Although it is cultivated almost all over the country, its industrial processing is limited to certain localities (province of Segovia and of Soria) where the dust from the root may produce an occupational susceptibility. This occurred in the following case, the only one of its kind in our records.

CASE 8.—F. L., thirty-eight years of age, employed in a chicory factory at Cuellar (Segovia). She had suffered paroxysms of asthma for over a year, these appearing almost daily, night or day. She observed that her health improved on keeping away from the factory. She also noted that the dust from the chicory produced nasal itching, sneezing, and spasmodic coughing and finally severe attacks of asthma which obliged her to leave her work. The patient, after three days in Madrid, remained free from symptoms. Examination of her respiratory apparatus was negative. All skin reactions including intradermals were also negative except that to chicory extract. This gave a strongly positive reaction (pseudopod infiltration the size of a coin of one peseta with extensive hyperemic halo). An attack of asthma followed the test. Passive transfer was obtained. She was advised to abandon her work, and up till now she remains in good health.

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STUDIES ON THE RELATION OF THE ADRENAL GLANDS TO ALLERGIC PHENOMENA

II. The Therapeutic Effect of Strychnin Stimulation of the Adrenal Medulla in Asthma

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AS A RESULT of the demonstrations by Stewart and Rogoff[1] that strychnin stimulation of the adrenal gland resulted in an increase in the output of epinephrine greater than the dose usually given for relief of attacks of asthma, and that this increase in output was maintained for several hours without depletion of the chromaffin tissue of the gland, and by Cohen, Rudolph, Wasserman and Rogoff[2] that there is no significant disturbance in the output of epinephrine from the adrenal medulla in dogs during anaphylactic shock, strychnin was administered to a series of patients to determine its efficiency in attacks of asthma.

The patients used in this study were all chronic asthmatic persons who were using from two to six subcutaneous doses of 0.4 c.c. of a 1:1,000 solution of epinephrine hydrochloride daily to control their attacks. Inasmuch as the increase in the epinephrine output in animals occurred only after hypertonicity was produced by strychnin, doses were given which would be expected to produce hypertonicity in man. This was considered quite safe since it has been shown that the effects of an overdose of strychnin can be controlled by a suitable dose of any one of the barbiturates.

In all the experiments, the patient having an attack was allowed to sit quietly for fifteen minutes to compensate for any increase in symptoms due to exercise in getting to this office.

Following this rest period, in the first two experiments, 1/6 gr. of strychnin sulphate was injected into the deltoid muscle and 1½ gr. of pentobarbital dissolved in distilled water into the opposite one. During an hour's observation there was no change in the symptoms. However, neither patient showed any evidence of hypertonicity, the two drugs having balanced each other's action.

Since hypertonicity was deemed necessary for the success of the experiments, the next four patients received doses of strychnin of from 1/12 gr. to 1/6 gr. intramuscularly without pentobarbital. All developed moderate to marked hypertonicity within thirty minutes. The

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attacks of asthma subsided slowly, and at the end of one hour one patient was completely free of symptoms and the others had only mild wheezing. By this time, however, the strychnin effect was so pronounced that the slightest exertion excited reflex spasm of the respiratory muscles and extreme dyspnea followed. The apparent relief of symptoms as well as the exacerbation on rising or walking paralleled the dose and the hypertonicity so that it was not possible to find a dose of strychnin which would relieve the asthmatic attack without making the patient worse shortly thereafter.

One patient was given strychnin sulphate $\frac{1}{20}$ gr. every four hours for four days without any effect on the asthmatic attack as measured by the number of epinephrine doses needed in each twenty-four-hour period to control his symptoms.

SUMMARY

Strychnin sulphate was administered to seven asthmatic patients with partial relief of the acute paroxysm in those who became hypertonic. The good effect of the strychnin was overshadowed by the complicating hypertonicity.

The administration of strychnin for the relief of attacks of asthma while experimentally sound is not useful clinically.

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A NEW TOURNIQUET AND AN IMPROVED TESTING NEEDLE*

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TOURNIQUET

THE importance of a tourniquet in the equipment of the general medical practitioner is recognized. To the allergist it is an essential part of his worktable make-up, ranking second in importance to adrenalin. In the ordinary routine of testing and treating the hypersensitive individual, the use of a tourniquet has been discussed in many articles on the diagnosis and treatment of the various hypersensitive

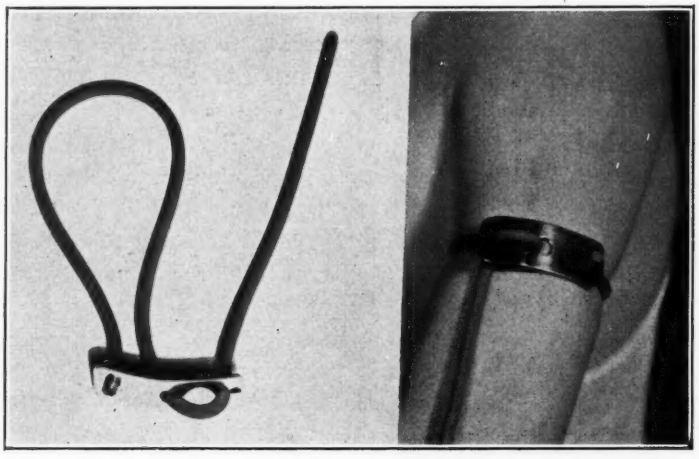

Fig. 1.—Two views of tourniquet.

conditions. In the treatment of the constitutional reaction its usefulness is generally recognized; indeed, in the treatment of hay fever, the application of a tourniquet before giving the injection is advocated as a prophylactic measure to prevent such reactions. It is, therefore, a necessity and should always be at hand; when applied, it should not slip, and should easily be released.

The tourniquet consists of a length of round solid rubber, one-quarter inch in diameter, which forms a loop with a chrome metal plate arched to fit the curve of the arm or leg (Fig. 1). The plate has two perforations for the rubber and is slit at the side so that the rubber, when stretched, easily slips in and is tightly held in place when relaxed. To release, the rubber is again stretched and slips out of the metal slit.

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The advantages of this tourniquet are that it is easily applied to the arm or leg and is easily tightened and fixed; that it is released by a simple pull of the rubber and can be manipulated with one hand; and that it contains no springs or accessory parts.

CURVED TESTING NEEDLE

The curved testing needle is offered to simplify the intracutaneous test in any of its uses, whether in allergy or in general practice. The ordinary needle now in use consists of a length of tubular shaft held firmly by a metal hub, either round or square, which fits upon the

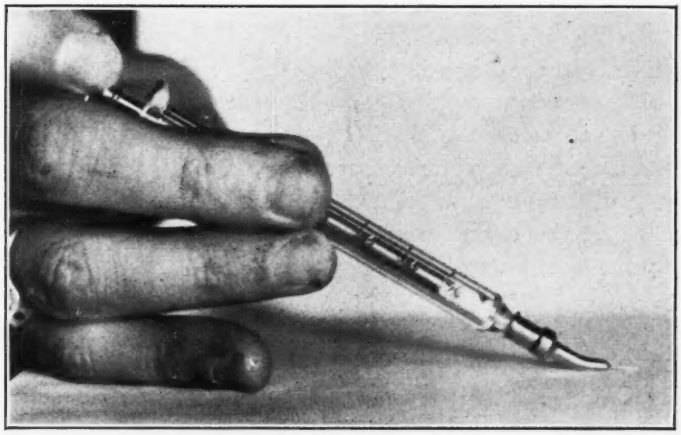

Fig. 2.—Curved needle.

syringe. To insert intracutaneously, the needle is held parallel to the skin, but in some regions (back) this is quite difficult, while in other regions the protruding part of the hub interferes.

The needle shown in Fig. 2 has a typical rounded hub with an elongated tapering point which holds one-quarter inch length of stainless, rustproof tubular shaft. Just above this point of insertion the hub is curved so that in testing the needle assumes a direction parallel to the skin, and an intracutaneous injection becomes quite simple in any part of the body. It has proved especially useful in testing the skin of the back (interscapular region) and also in testing in hypersensitive skin where pressure of the hub of the needle results in the formation of pressure wheals.

Society Proceedings

REPORT OF THE JOINT COMMITTEE ON STANDARDS

This Committee was established in 1931 by the Society for the Study of Asthma and Allied Conditions and the Association for the Study of Allergy. Since then, each member of each society has been invited to submit to the Committee the data concerning the hospital allergy clinics with which he is connected. Special questionnaire forms were provided for this purpose, covering all phases of clinic activity, and the qualifications of the clinic personnel. Five sample case records were submitted by each clinic. In each instance, each member of the Committee passed independently on all the material, and the votes for approval or rejection were always unanimous.

At its meeting in New York on December 8, 1934, the Committee voted to publish in the JOURNAL OF ALLERGY, the official organ of the two societies, a list of the clinics which fulfilled the minimum requirements adopted by the Committee.

The Committee plans to publish such a list of approved clinics annually in the JOURNAL OF ALLERGY. It is further planned that in the program of the annual meeting of each Society there shall appear a notice that members wishing to submit data of hospital allergy clinics to the Committee for approval should communicate with the secretary of the Committee.

The Committee realizes that it has no jurisdiction over any hospital allergy clinic. It, therefore, does not publish the names of clinics that have been disapproved. For the same reason, the Committee has thus far not solicited the submission of clinic data by any one except members of the Association for the Study of Allergy or the Society for the Study of Asthma and Allied Conditions.

The Committee nevertheless feels that in passing judgment upon allergy clinics it is performing a service of distinct value to hospitals and to the medical profession generally. It, therefore, invites any hospital allergy clinic to submit its data for approval by the Committee. If approved, the name of the clinic will be published in this JOURNAL in the annual list. For further information, please communicate with the Secretary.

For the Society for the Study of Asthma and Allied Conditions:

J. ALEXANDER CLARKE, JR., Philadelphia
ROBERT A. COOKE, New York
LESLIE N. GAY, Baltimore

For the Association for the Study of Allergy:

RICHARD A. KERN, University Hospital, Philadelphia (Secretary)
FRANCIS M. RACKEMANN, Boston
WARREN T. VAUGHAN, Richmond

List of Hospitals With Allergy Clinics That Have Been Approved by the Joint Committee on Standards for the Association for the Study of Allergy and the Society for the Study of Asthma and Allied Conditions

ARIZONA

Phoenix

1. St. Luke's Home

CALIFORNIA

Los Angeles

2. Children's Hospital
3. Los Angeles County General Hospital
4. Santa Fe Coast Lines Hospital

MARYLAND

Baltimore

5. Johns Hopkins Hospital

MASSACHUSETTS

Boston

6. Beth Israel Hospital
7. Massachusetts General Hospital

MINNESOTA

Rochester

8. Mayo Clinic

MISSOURI

St. Louis

9. Barnes Hospital
10. McMillan Hospital

NEW JERSEY

Montclair

11. Mountainside Hospital

NEW YORK

Brooklyn

12. Jewish Hospital

New York

13. Beth Israel Hospital
14. French Hospital
15. Gouverneur Hospital
16. Lebanon Hospital
17. Midtown Hospital
18. New York Post-Graduate Hospital
19. Roosevelt Hospital
20. St. Luke's Hospital
21. Sydenham Hospital
22-25. University and Bellevue Hospital (Four allergy clinics of this hospital have been approved)

Rochester

26. Rochester General Hospital
27. Strong Memorial Hospital

PENNSYLVANIA

Philadelphia

28. Graduate Hospital of the University of Pennsylvania
29. Hospital of the University of Pennsylvania
30. Jefferson Hospital
31. Mount Sinai Hospital
32. Pennsylvania Hospital
33. Philadelphia General Hospital
34. Temple University Hospital

Pittsburgh

35. University of Pittsburgh Hospital Falk Clinic.

Department of Reviews and Abstracts

Selected Abstracts

Clinical Data in Asthma. Witts, L. J.: Lancet **226**: 275, 1934.

Observations were made on 500 patients with asthma investigated at the asthma research clinic at Guy's Hospital during the last four years; 350 of them were over fifteen years of age. During the same period collateral investigations were made on 100 people who presented none of the symptoms of asthma or allied disease. Studies were made on the nature of the paroxysms, the sputum and blood, response to adrenalin, skin reactions, and other clinical features. This investigation confirms the conventionally accepted data in most respects. The association of asthma and tuberculosis and of asthma and rheumatism was very rarely encountered. Moreover, bacterial allergy, of the type which is believed to give rise to rheumatism, carditis and acute nephritis, was found to occur rarely with asthma. A most striking difference between the asthmatic individual and the normal person was the frequency of lesions of the upper and lower respiratory tracts.

A Study of the Psychophysical Asthma Problem. Hanse, A.: Nervenarzt **7**: 17, 1934.

Forty cases of true asthma were studied from various standpoints, particularly from that of the patient's biological constitution as well as from a structural or anthropologic aspect. A definite linkage of such individuals to a uniform body type and a corresponding characteristic biological configuration cannot be proved. Two make-ups are encountered, the tetanoid (spastic) and the parasympathetic, which together are described as the so-called "T-type." These accounted for 29 cases. The remaining 11 showed a heightened sympathetic response and a definite psychogenic make-up. In this second group, however, spastic tendencies were noted about the time that paroxysms occurred, especially an increased excitability of the muscles. In asthmatic individuals, then, the situation is complicated. In the T-type of individual one may look upon the attack as a typical spastic manifestation. In the sympathicotonic types the asthmatic paroxysm is considered a conditioned reflex. The principles of therapy are to employ antispastic measures as well as desensitization in the T-types, and in addition to use psychotherapy in the second group.

Asthmatic Breathing as Recorded by the Kymogram. Weltz, G. A., and Van Niekerk, J.: Fortschr. a. d. Geb. d. Röntgenstrahlen **48**: 564, 1934.

It can be shown that breathing during an asthmatic paroxysm is no different than that recorded with bronchial stenosis, no matter whether the attack is due to a true allergic cause or to extrinsic factors. Through increased tension of the muscles of inspiration which result from a supplementary inspiration reflex, lung distention occurs. This phenomenon supports the hypothesis that the bronchi through contraction and relaxation of their musculature take an active part in maintaining lung ventilation. During severe asthmatic paroxysms the extreme bronchial narrowing may markedly interfere with the pulmonary circulation.

Intracutaneous Reaction to Carcinoma. Pichler, K.: Klin. Wchnschr. **13**: 451, 1934.

Fresh specimens of carcinoma were macerated and a lipoid was extracted from them as follows: The material was acidified with 20 per cent phosphoric acid and then extracted with ethyl ether. The ether was filtered off and the residue further extracted. The ether extract was evaporated in a vacuum. The recovered lipoid was suspended to 1 per cent concentration in 0.3 per cent tricresol and neutralized with sodium carbonate.

This lipoid carcinoma extract was sterilized and used for intracutaneous tests on 40 patients. Of these, 15 had a known carcinoma and the remaining 22 were used as controls. The carcinomatous subjects all gave positive skin reactions to the lipoid extract, whereas in the controls only two gave doubtfully positive, the others negative responses.

Pollen Sensitiveness in Children With Asthma. Pearson, R. B.: Arch. Dis. Child. **9**: 353, 1934.

The frequency of pollen sensitization in asthmatic children was determined by testing 250 unselected patients attending an asthma clinic. It was found that 42 per cent of the children gave a positive reaction to grass pollen; whereas in a control group of 33 nonasthmatic children none gave positive tests to grasses. Pollen sensitiveness increased after the age of nine years. The highest proportion giving pollen skin reactions was found in those patients in whom allergic eczema was or had been present. These included 20 per cent of the series. This group showed, however, a capacity for sensitization to all allergens tested. The group of patients with pure spasmodic asthma gave the next highest incidence of pollen reactions; whereas those in whom asthma followed

respiratory infection gave the least. It is believed that grass pollen is of greater importance in the precipitation of asthmatic attacks than is generally recognized.

"Pseudocholecystitis" Apparently Caused by Food-Sensitiveness. Alvarez, W. C.: Proc. Staff Meet. Mayo Clin. **9**: 680, 1934.

Four patients who had symptoms typical of gallstone colic are cited. Two of these were operated upon, and despite the fact that dense adhesions were found around the gallbladder, symptoms returned immediately upon resuming a moderate post-operative diet. The other two patients showed no pathologic changes referable to the gallbladder on laboratory examinations. All four became much improved after proper diet eliminations had been effected.

A Simple Apparatus for Determining the Pollen Content of the Air. Durham, O. C.: Aerologist **11**: 10, 1935.

Although the gravity method for determining a continuous seasonal record of pollen counts is very satisfactory, the method is too slow where instantaneous counts are desired. A simple portable apparatus designed by Dr. E. Vernon Hill is described. This is a gyrating instrument consisting of a 20 inch shaft with a clip for holding a glass slide. The shaft is geared to a handle so that it rotates as the apparatus is whirled in the air. For each revolution of the shaft, the slide rotates three times. Thus, the air impinging the slide is not received at right angles but is rather cut by the slide at all possible angles. The apparatus was standardized by comparison with gravity exposures, and an equivalent of a twenty-four-hour exposure was found to be 1,930 turns of the apparatus.

A test of the pollen concentration at any given spot, whether indoors or out-of-doors, can be made within two minutes. The distribution of pollen from a definite source, such as a large tree in full bloom, may be studied at various distances and various directions; also the effect of unusual weather conditions, such as fog and rain, on the pollen content of the air may be easily studied. The contact of a pollen sensitive person may be investigated in great detail, as exposures may be made in his bedroom, office, yard, etc. A table is given showing great differences of concentration at nearby places within an hour or two.

Rapidity of Shrinkage and Immediate Secondary Reactions Following Local Applications of Ephedrine and Benzedrine. Scarano, J. A.: Med. Rec. **140**: 602, 1934.

Inasmuch as various synthetic compounds have been offered as substitutes for ephedrine, because of minor disadvantages such as stinging and discomfort following its use, the effectiveness of benzedrine (N.N.R.), benzyl, methyl carbinamine was tried. This was done by

studying the rapidity of shrinkage and the immediate and secondary reactions following the local application of ephedrine and of benzedrine applied in 1 per cent oil in two groups of 25 patients each, suffering from common rhinologic conditions.

Both groups gave maximum shrinkage of the nasal mucosa within five minutes. The rapidity of action was slightly greater with ephedrine. Unpleasant immediate reactions, such as stinging, burning, lacrimation, and sneezing, were considerably more marked and more persistent with ephedrine than with benzedrine. Secondary reactions, as turgescence, atony, and bogginess, were studied one hour after application and at two-day intervals throughout a weekly period. The secondary reactions following benzedrine were less severe and less frequent than those with ephedrine. The same held true when benzedrine was used in inhaler form.

Allergy to Chicle. Preliminary Report. Kleinman, A. I.: J. A. M. A. **104**: 455, 1935.

Two cases are cited wherein typical but severe symptoms of vasomotor rhinitis occurred soon after the patients began to chew gum. This material was suspected, and various substances of which chewing gum is composed were tested. Each gave a marked positive skin test to chicle. In 25 allergic controls only one moderately positive reaction was secured.

Skin Reaction to Tuberculin Proteins in Hodgkin's Disease. Steiner, C. P.: Arch. Int. Med. **54**: 11, 1934.

Tuberculin proteins from both human and avian strains were prepared. These were used intracutaneously on 35 patients with Hodgkin's disease and on 38 controls with other forms of lymphoma. No evidence of specific sensitiveness to avian tuberculin was obtained. A pronounced absence of sensitiveness to both tuberculin proteins was found in patients with Hodgkin's disease. The interpretation offered is that either the process of Hodgkin's disease desensitizes to these tuberculin proteins or the disease occurs in persons in whom the development of normal sensitization to tuberculin is impossible. The author states that it is difficult to conceive of either of these phenomena as occurring in a disease unrelated to tuberculosis.

Decrease of the Complement Content of the Blood in Allergic Disorders. Paul, B., and Pely, M.: Klin. Wchnschr. **14**: 163, 1935.

Recent investigations are cited which seem to indicate that the complement content of the blood is an expression of colloidal conditions. Since allergy is thought to be bound up with altered colloidal states, estimations of the complement content of the blood in allergic disorders were made. The normal amount of complement is considered that which

causes hemolysis of 0.5 c.c. of a 5 per cent suspension of sheep corpuscles by 0.5 c.c. of a given hemolysin. This averages 0.04 c.c. of serum. In allergic disorders the quantity of serum required is much larger than this, which tends to confirm the colloidal theory of allergy.

Allergic Inflammation of the Lungs. Fried, B. M.: Arch. Path. **18:** 865, 1934.

Rabbits were sensitized by repeated intraperitoneal injections of horse serum, the last injection (defined as "shocking") being introduced into the lungs via the trachea. A study of the lungs of these animals showed that the intratracheal ("shocking") injection of the heterologous serum soon produced an acute exudative, rapidly spreading inflammation in the lungs. In a high percentage of the animals studied, the lesion at its height was confined to one lung, having a diffuse lobar distribution. The gross and microscopic aspects of the pulmonary lesion resembled in many ways acute lobar (fibrinous or genuine) pneumonia as seen in man. The nature of the latter disease was analyzed, and the hypothesis was expressed that in this malady the character of the lesion in the lung probably depends on a state of local pulmonary hypersensitiveness (allergy, hyperergy). The similarities between the lesions in the lungs in genuine pneumonia in man and those observed in the experiments with horse serum reported in this article favor this hypothesis.